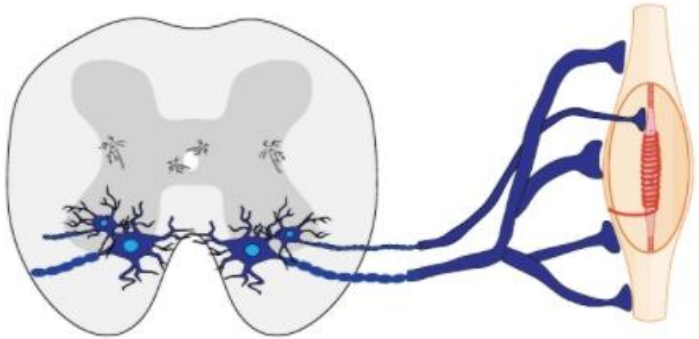
Single-cell transcriptomic analysis reveals genetic drivers of fast/slow motor neuron identity

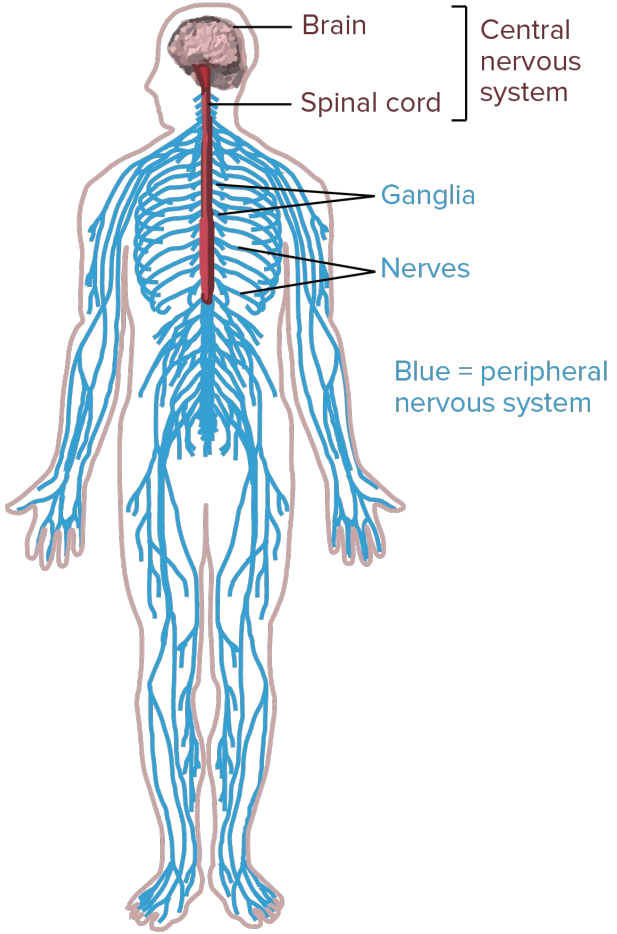
Luke Zhao (12) - RRI H21

What are motor neurons?

- Connect brain → muscles
- Project across whole body, innervate muscles
- Responsible for voluntary movement

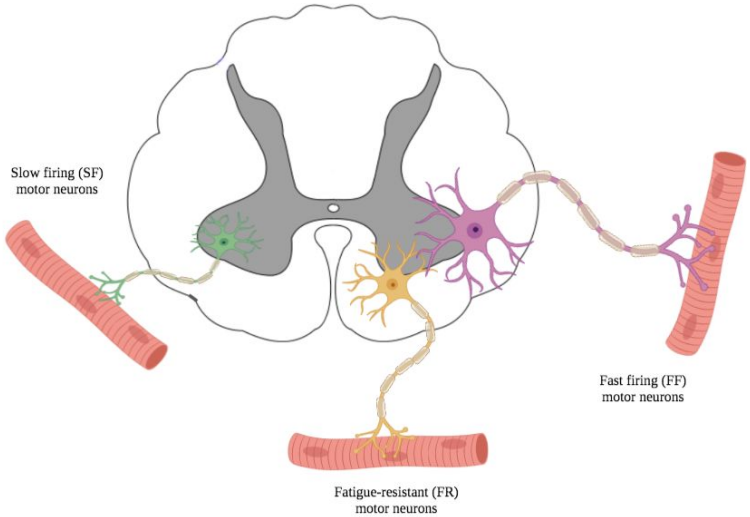


Blum et al. 2021



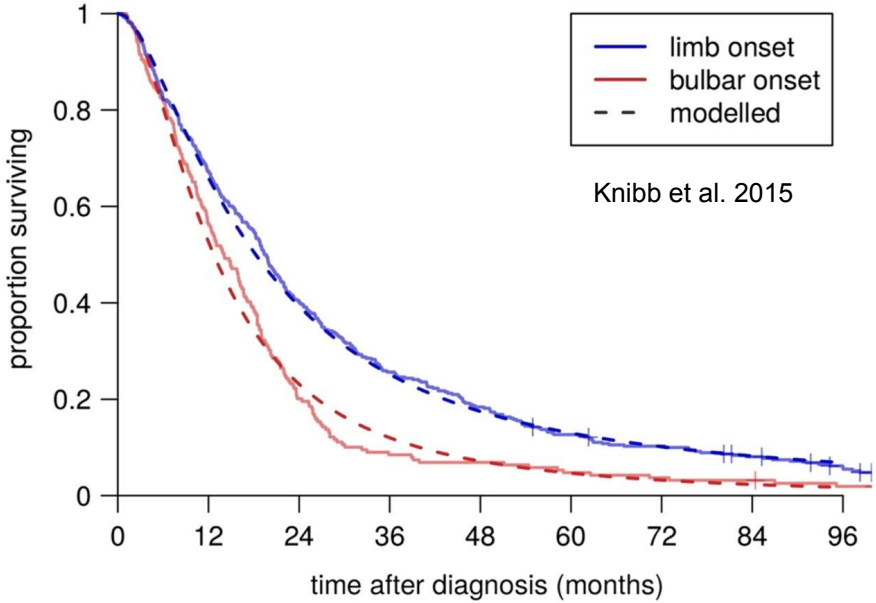
Types of motor neurons

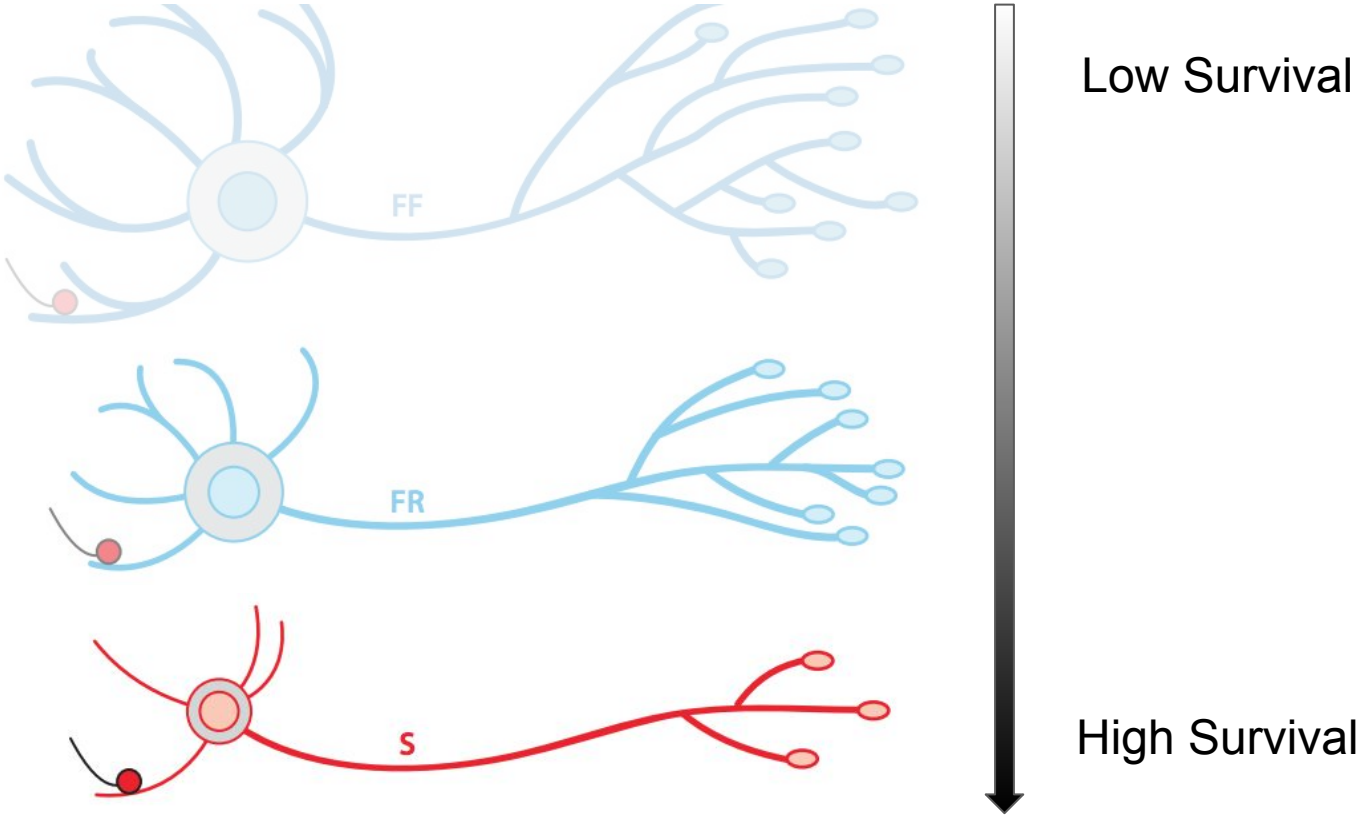
- Several types control voluntary movement
  - **Slow-firing (SF)** - control posture and endurance muscles
  - **Fatigue-resistant (FR)** - control intermediate muscles
  - **Fast-firing (FF)** - control fast, forceful muscle contractions



ALS

- Fatal, devastating neurodegenerative disease
- Rapid progression
- 1 in 400 lifetime risk of disease




FF
FR
S
Low Survival
High Survival

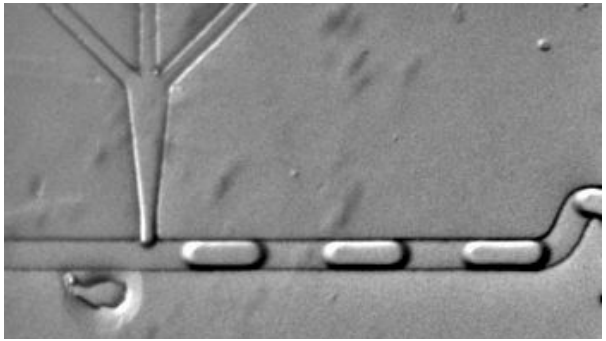
nature neuroscience

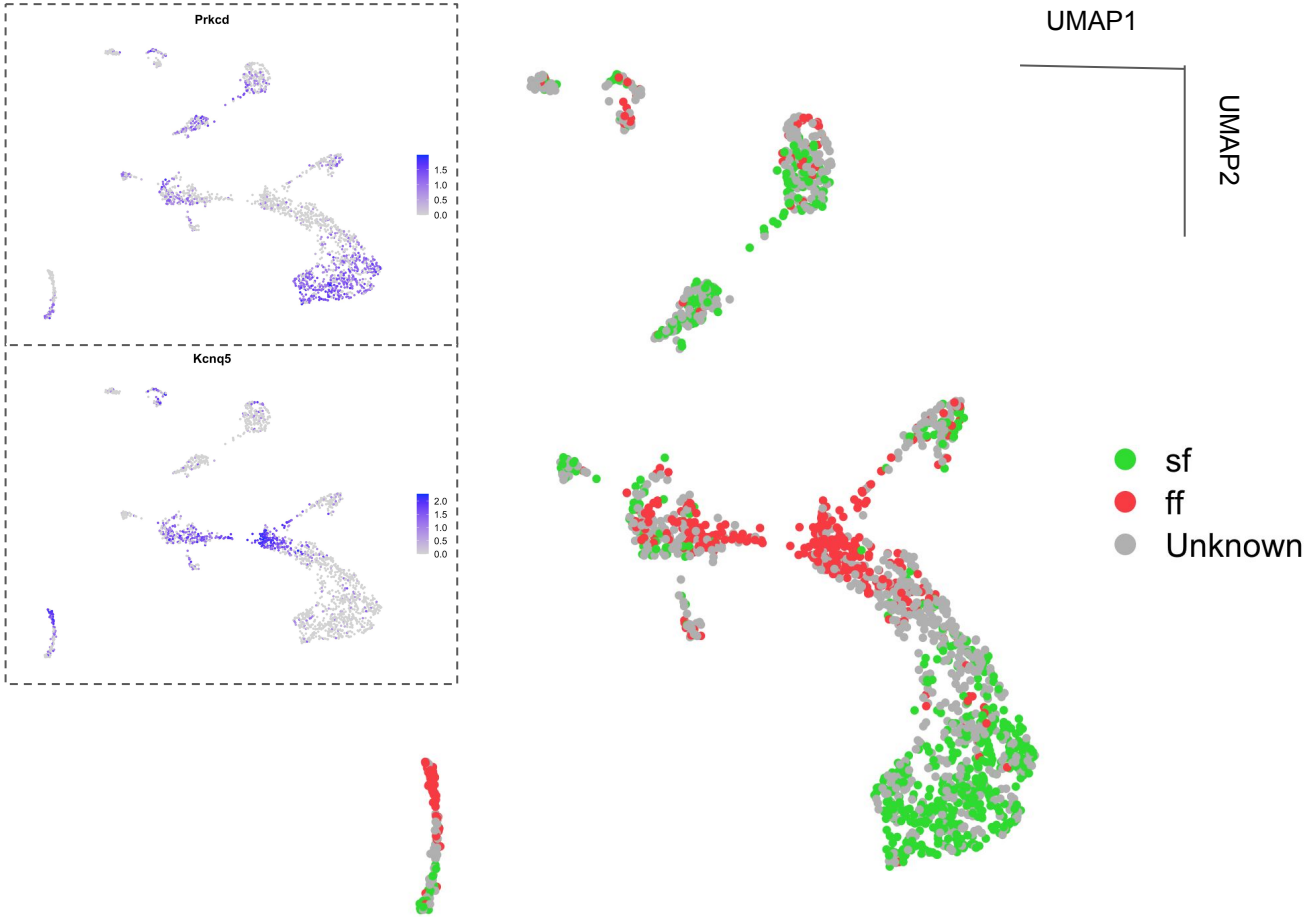
Resource | Published: 15 February 2021

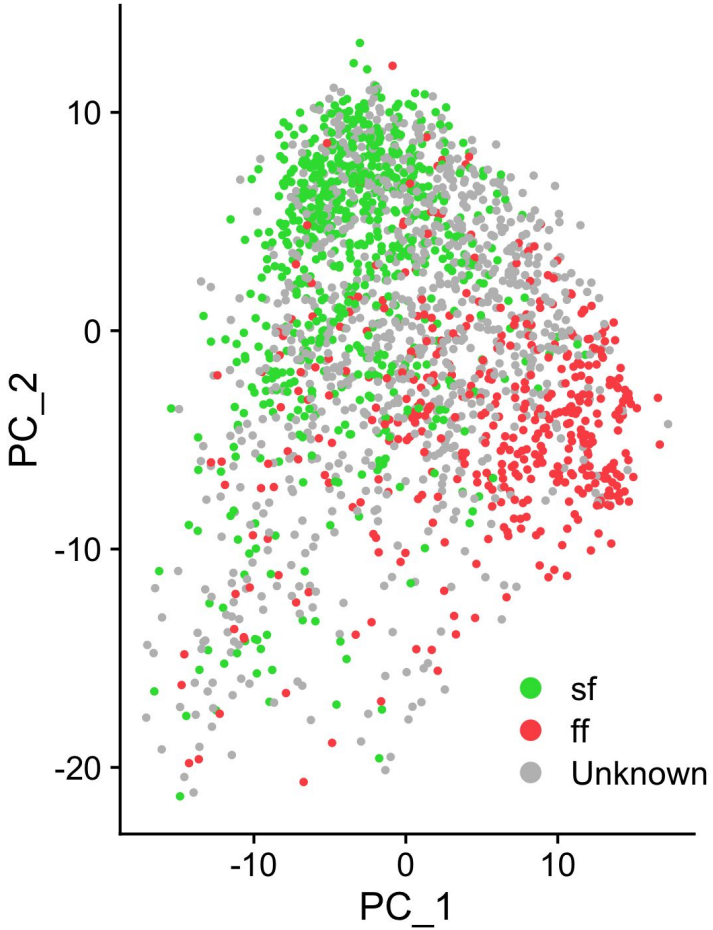
Single-cell transcriptomic analysis of the adult mouse spinal cord reveals molecular diversity of autonomic and skeletal motor neurons

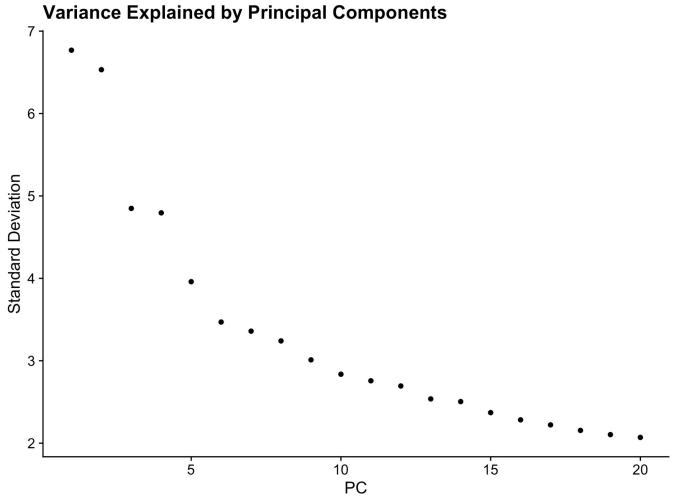
Jacob A. Blum, Sandy Klemm, Jennifer L. Shadrach, Kevin A. Guttenplan, Lisa Nakayama, Arwa Kathiria, Phuong T. Hoang, Olivia Gautier, Julia A. Kaltschmidt, William J. Greenleaf & Aaron D. Gitler

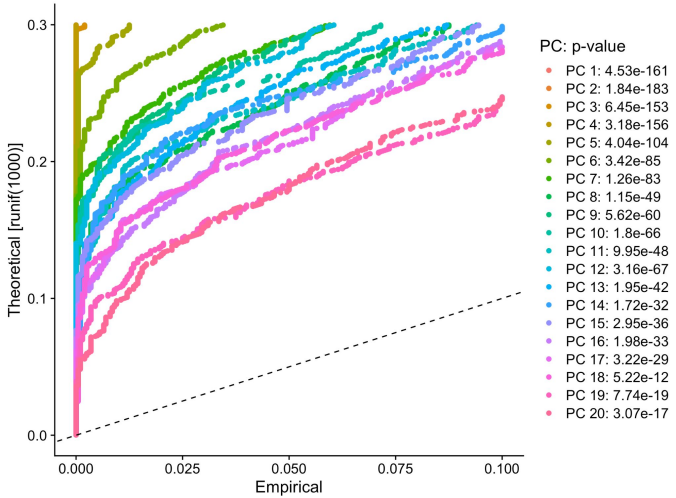
Nature Neuroscience **24**, 572–583 (2021) | Cite this article

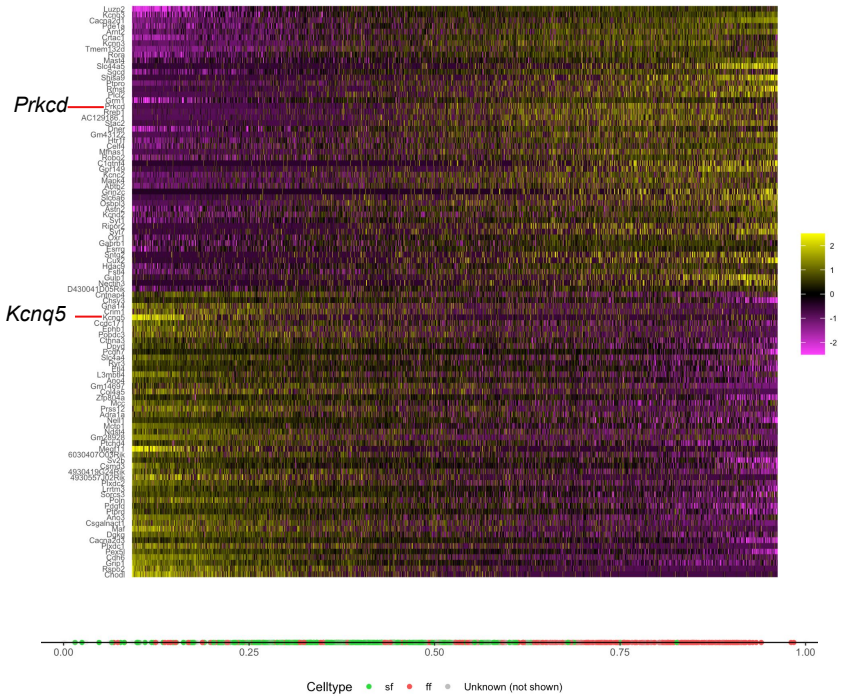


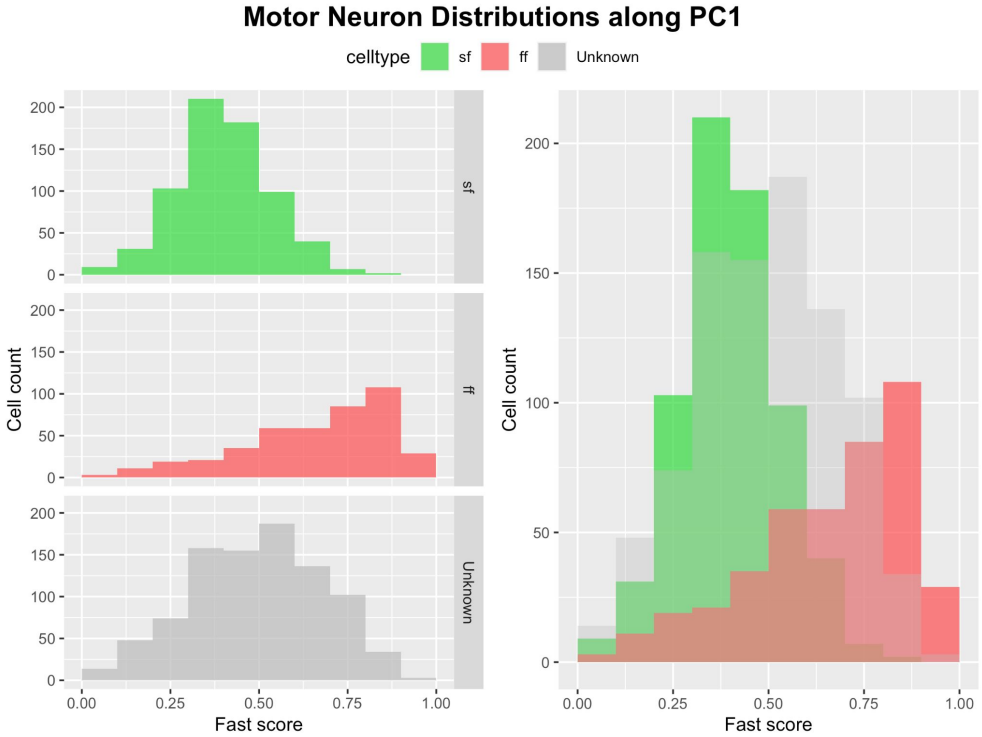

Prkcd
1.5
1.0
0.5
0.0
Kcnq5
2.0
1.5
1.0
0.5
0.0
UMAP1
UMAP2
sf
ff
Unknown

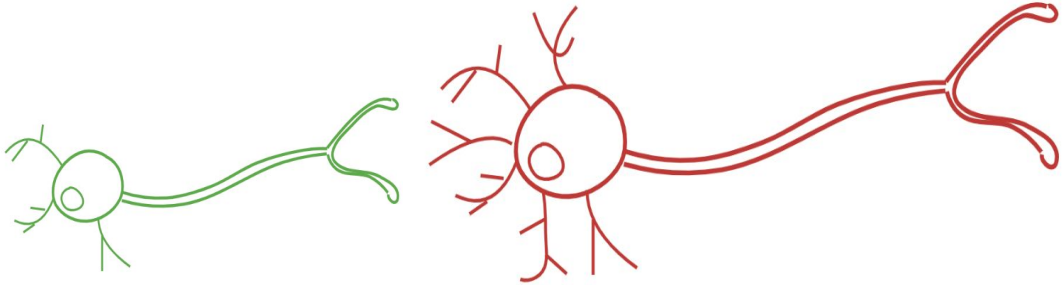

10
0
-10
-20
PC_2
-10
0
10
PC_1
sf
ff
Unknown


Variance Explained by Principal Components
Standard Deviation
7
6
5
4
3
2
5
10
15
20
PC


Theoretical [runif(1000)]
0.3
0.2
0.1
0.0
0.000
0.025
0.050
0.075
0.100
Empirical
PC: p-value
PC 1: 4.53e-161
PC 2: 1.84e-183
PC 3: 6.45e-153
PC 4: 3.18e-156
PC 5: 4.04e-104
PC 6: 3.42e-85
PC 7: 1.26e-83
PC 8: 1.15e-49
PC 9: 5.62e-60
PC 10: 1.8e-66
PC 11: 9.95e-48
PC 12: 3.16e-67
PC 13: 1.95e-42
PC 14: 1.72e-32
PC 15: 2.95e-36
PC 16: 1.98e-33
PC 17: 3.22e-29
PC 18: 5.22e-12
PC 19: 7.74e-19
PC 20: 3.07e-17


Prkcd
Kcnq5
0.00
0.25
0.50
0.75
1.00
Celltype
sf
ff
Unknown (not shown)


Motor Neuron Distributions along PC1
celltype
sf
ff
Unknown
Cell count
Fast score
0.00
0.25
0.50
0.75
1.00
0
50
100
150
200



Survival in ALS

Postsynaptic density

Firing rate

Action potential threshold

Cell size

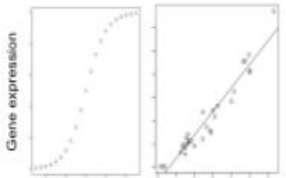
Dendritic branching

Axon diameter

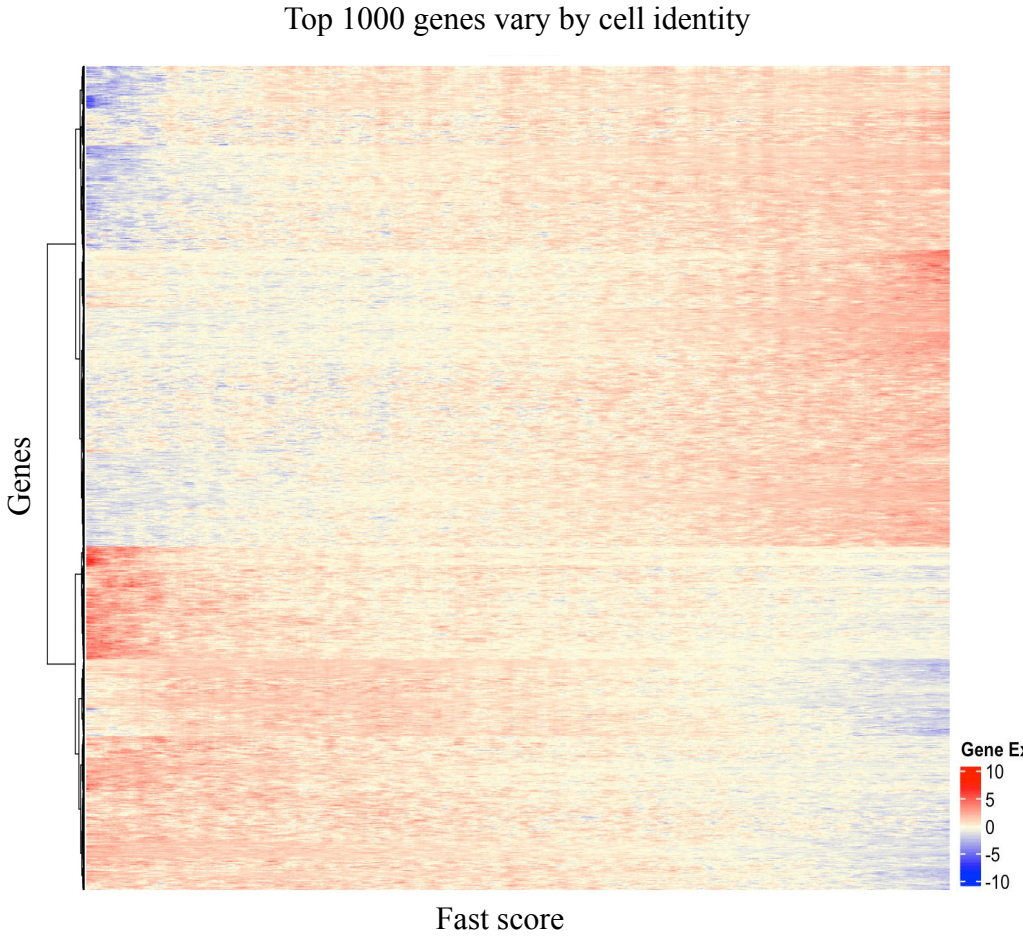
Resting membrane potential

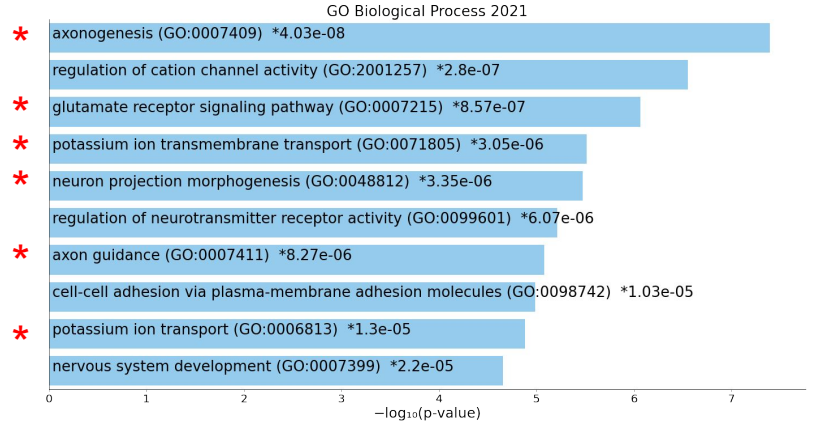
Regression analysis: fitting linear models to gene expression

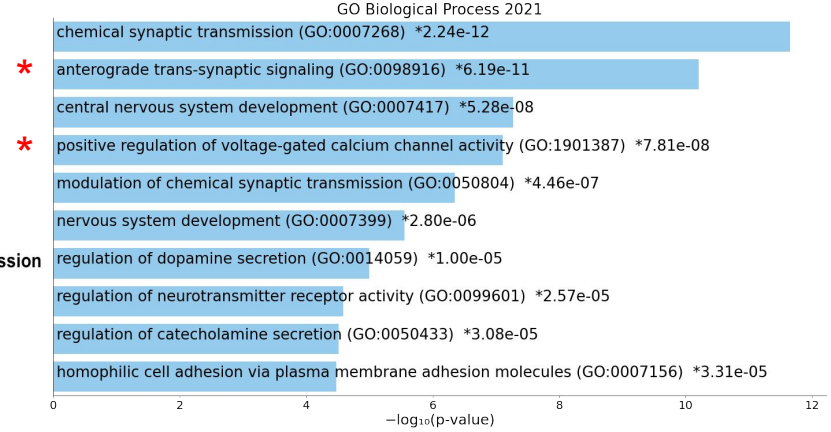
$$log(y_i) = \beta_0 + \beta_t x_t$$

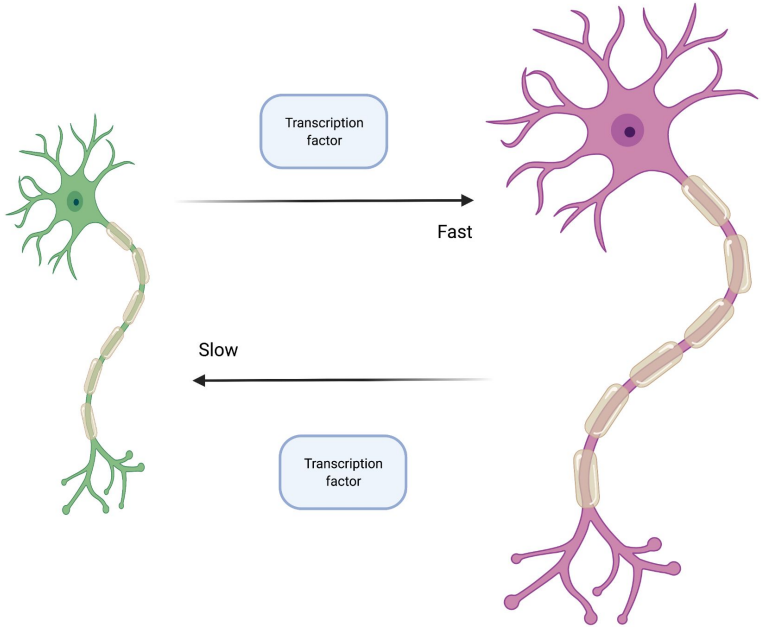


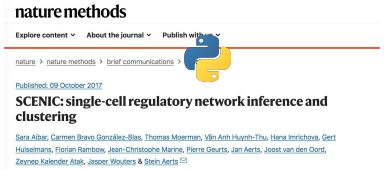
1502 genes change significantly with fast score


Top 1000 genes vary by cell identity
Genes
Fast score
Gene Expression
10
5
0
-5
-10


GO Biological Process 2021
axonogenesis (GO:0007409) *4.03e-08
regulation of cation channel activity (GO:2001257) *2.8e-07
glutamate receptor signaling pathway (GO:0007215) *8.57e-07
potassium ion transmembrane transport (GO:0071805) *3.05e-06
neuron projection morphogenesis (GO:0048812) *3.35e-06
regulation of neurotransmitter receptor activity (GO:0099601) *6.07e-06
axon guidance (GO:0007411) *8.27e-06
cell-cell adhesion via plasma-membrane adhesion molecules (GO:0098742) *1.03e-05
potassium ion transport (GO:0006813) *1.3e-05
nervous system development (GO:0007399) *2.2e-05
0
1
2
3
4
5
6
7
−log10(p-value)


GO Biological Process 2021
chemical synaptic transmission (GO:0007268) *2.24e-12
anterograde trans-synaptic signaling (GO:0098916) *6.19e-11
central nervous system development (GO:0007417) *5.28e-08
positive regulation of voltage-gated calcium channel activity (GO:1901387) *7.81e-08
modulation of chemical synaptic transmission (GO:0050804) *4.46e-07
nervous system development (GO:0007399) *2.80e-06
regulation of dopamine secretion (GO:0014059) *1.00e-05
regulation of neurotransmitter receptor activity (GO:0099601) *2.57e-05
regulation of catecholamine secretion (GO:0050433) *3.08e-05
homophilic cell adhesion via plasma membrane adhesion molecules (GO:0007156) *3.31e-05
0
2
4
6
8
10
12
−log10(p-value)


Transcription factor
Fast
Slow
Transcription factor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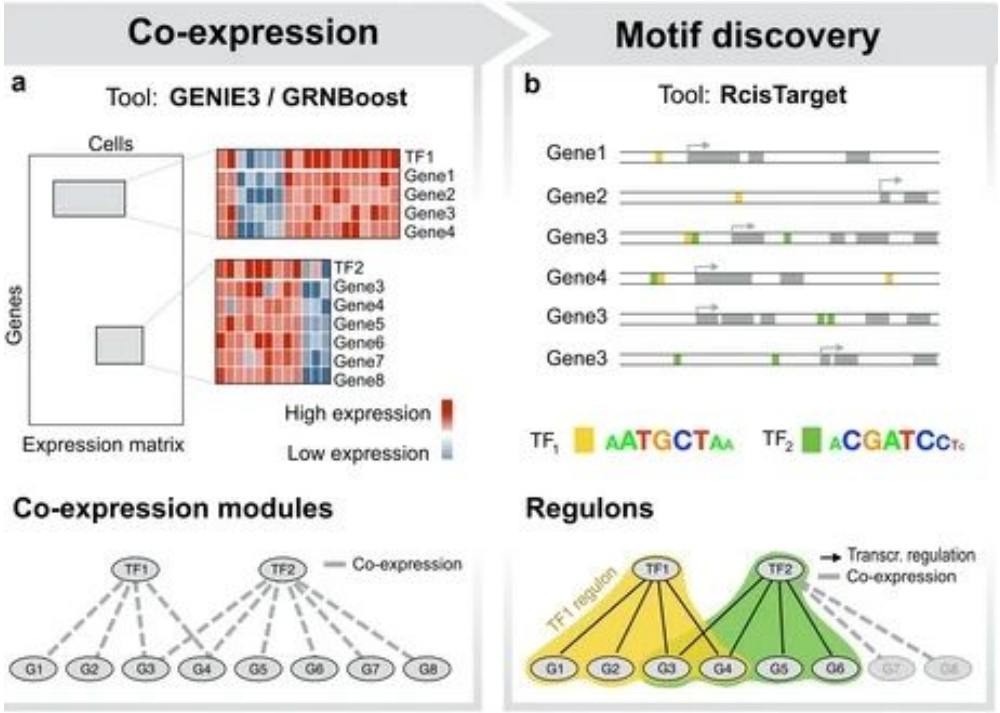
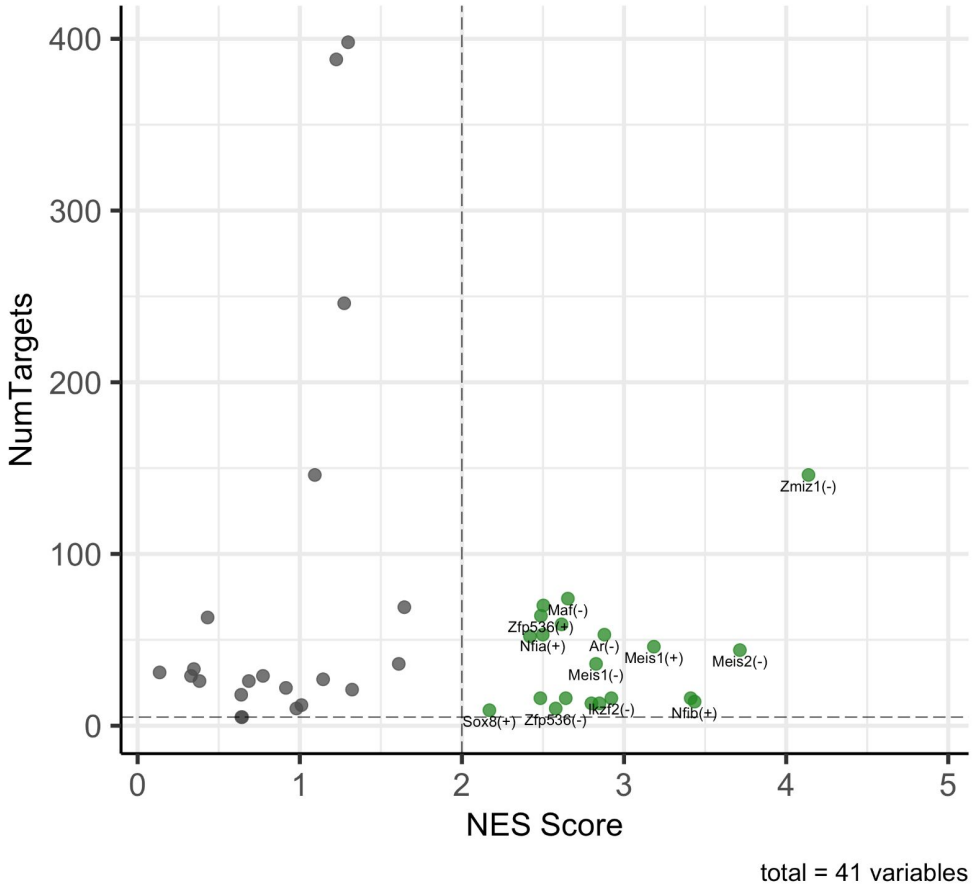
SCENIC: single-cell regulatory network inference and clustering
Sara Aibar, Carmen Bravo González-Blas, Thomas Moerman, Vân Anh Huynh-Thu, Hana Imrichova, Gert Hulselmans, Florian Rambow, Jean-Christophe Marine, Pierre Geurts, Jan Aerts, Joost van den Oord, Zeynep Kalender Atak, Jasper Wouters & Stein Aerts

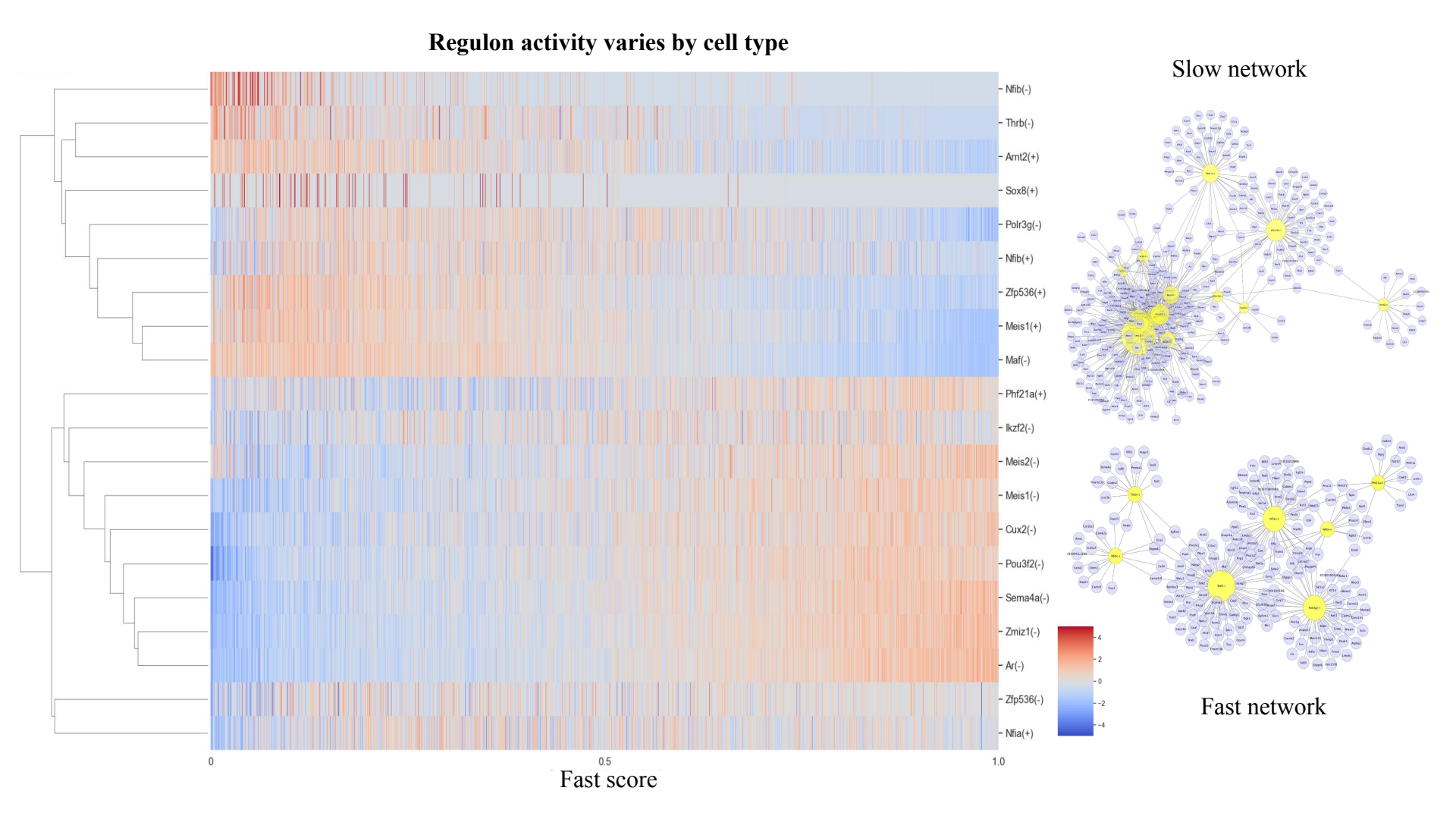

Co-expression
Motif discovery
a
Tool: GENIE3 / GRNBoost
Cells
Genes
Expression matrix
TF1
Gene1
Gene2
Gene3
Gene4
TF2
Gene3
Gene4
Gene5
Gene6
Gene7
Gene8
High expression
Low expression
b
Tool: RcisTarget
Gene1
Gene2
Gene3
Gene4
Gene3
Gene3
TF1
TF2
Co-expression modules
TF1
TF2
Co-expression
G1
G2
G3
G4
G5
G6
G7
G8
Regulons
TF1 regulon
TF1
TF2
Transcr. regulation
Co-expression
G1
G2
G3
G4
G5
G6

Motor Neuron Transcription Factors

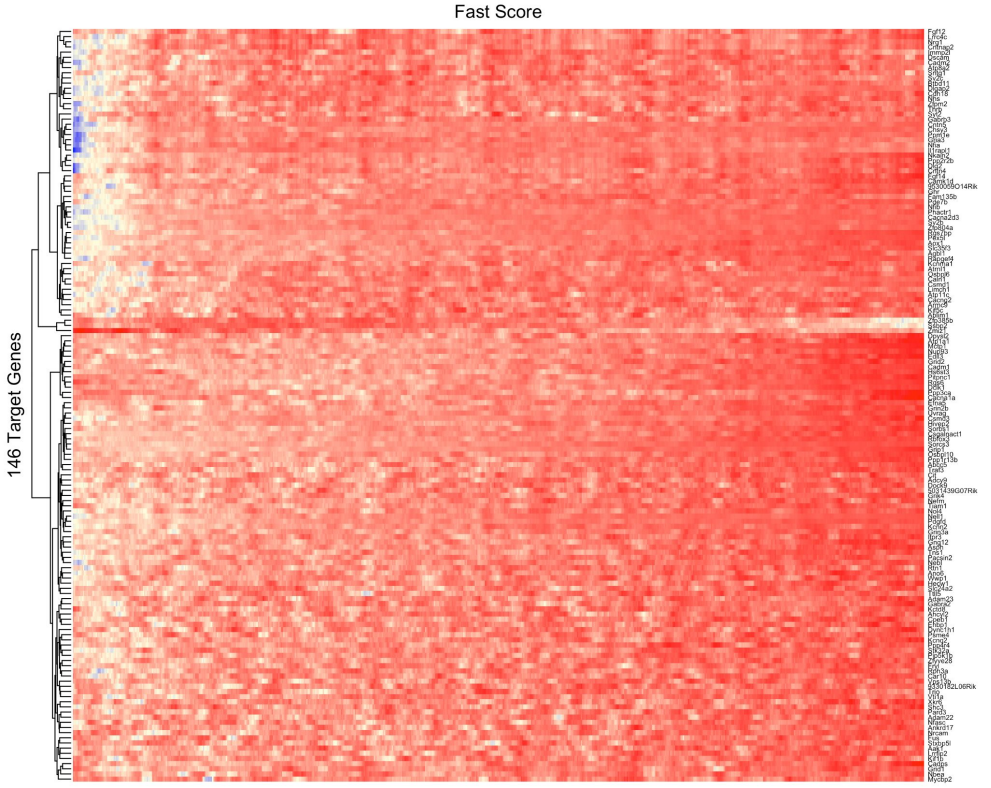
Divided by confidence (NES) score and target number

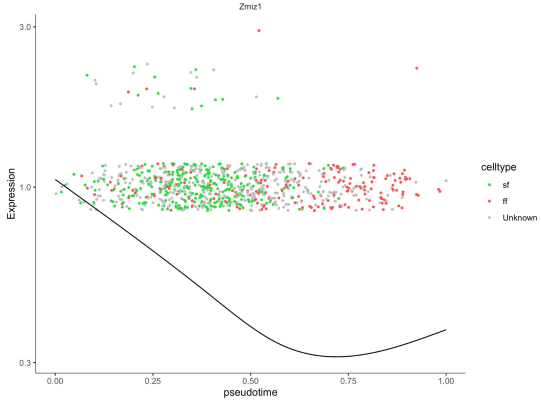


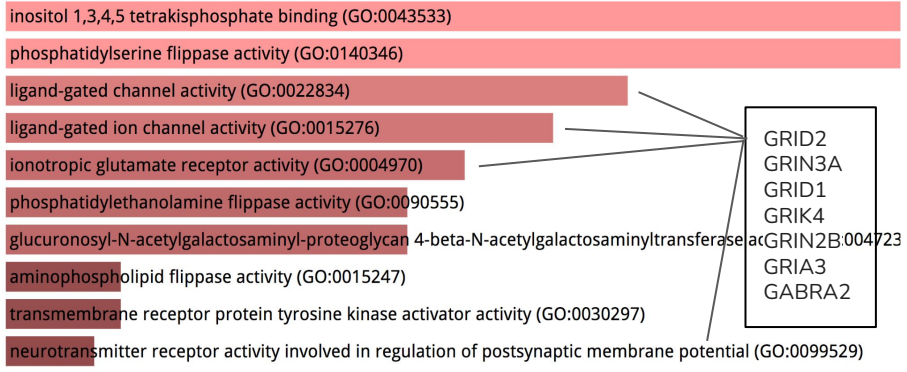
#	TF	Score	Targets
...			
0	Zmiz1(-)	4.137666	146
1	Meis2(-)	3.714377	44
2	Nfib(+)	3.435139	14
3	Nfib(-)	3.411004	16
4	Meis1(+)	3.184089	46
5	Ikzf2(-)	2.921785	16
6	Ar(-)	2.878836	53
7	Phf21a(+)	2.848180	13
8	Meis1(-)	2.827271	36
9	Cux2(-)	2.798850	13
10	Maf(-)	2.653498	74
11	Thrb(-)	2.640621	16
12	Pou3f2(-)	2.614854	59
13	Zfp536(-)	2.578397	10
14	Sema4a(-)	2.502338	70
15	Nfia(+)	2.498672	53
16	Zfp536(+)	2.487250	64
17	Arnt2(+)	2.483991	16
18	Polr3g(-)	2.420024	52
19	Sox8(+)	2.169521	9


Regulon activity varies by cell type
Nfib(-)
Thrb(-)
Arnt2(+)
Sox8(+)
Polr3g(-)
Nfib(+)
Zfp536(+)
Meis1(+)
Maf(-)
Phf21a(+)
Ikzf2(-)
Meis2(-)
Meis1(-)
Cux2(-)
Pou3f2(-)
Sema4a(-)
Zmiz1(-)
Ar(-)
Zfp536(-)
Nfia(+)
0
0.5
1.0
Fast score
4
2
0
−2
−4
Slow network
Fast network

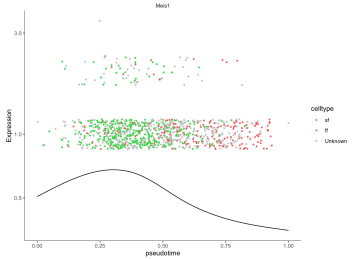
Zmiz1 - repressor of fast identity

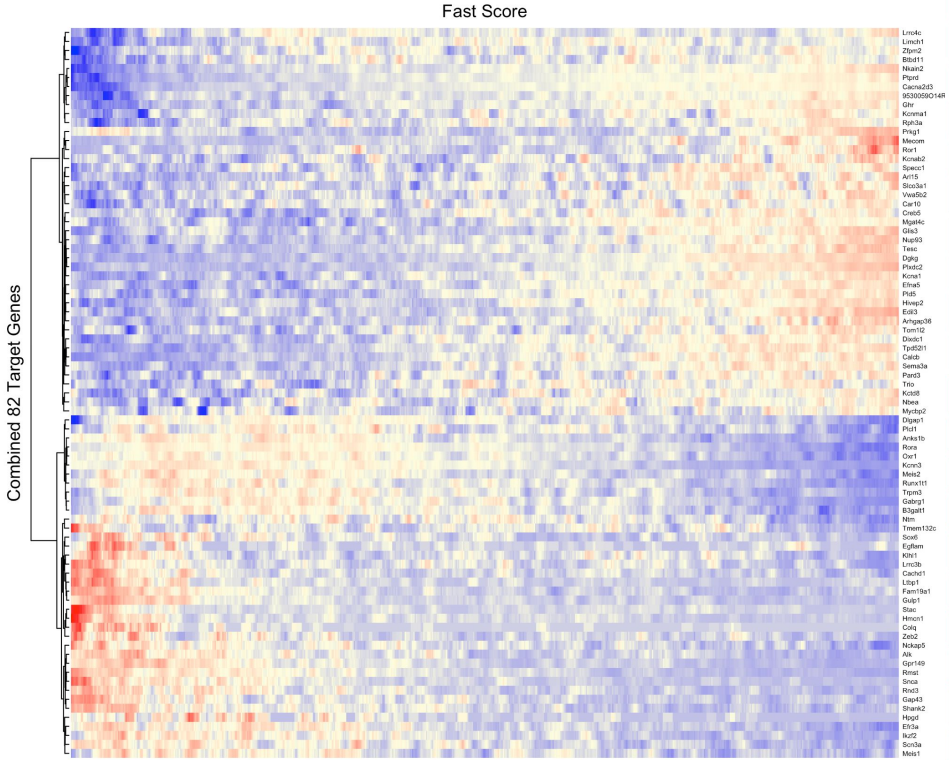






Meis1 - repressor of fast identity, activator of slow identity





neurotrophin TRK receptor binding (GO:0005167)

neurotrophin TRKA receptor binding (GO:0005168)

transmembrane receptor protein tyrosine kinase activator activity (GO:0030297)

histone methyltransferase activity (H3-K9 specific) (GO:0046974)

phosphate ion binding (GO:0042301)

inositol 1,4,5 trisphosphate binding (GO:0070679)

diacylglycerol kinase activity (GO:0004143)

calcium-activated potassium channel activity (GO:0015269)

sodium-independent organic anion transmembrane transporter activity (GO:0015347)

voltage-gated cation channel activity (GO:0022843)

prostaglandin E receptor activity (GO:0004957)

coreceptor activity involved in Wnt signaling pathway, planar cell polarity pathway (GO:1904929)

cyclic nucleotide-dependent protein kinase activity (GO:0004690)

oxysterol binding (GO:0008142)

beta-1,3-galactosyltransferase activity (GO:0048531)

coreceptor activity involved in Wnt signaling pathway (GO:0071936)

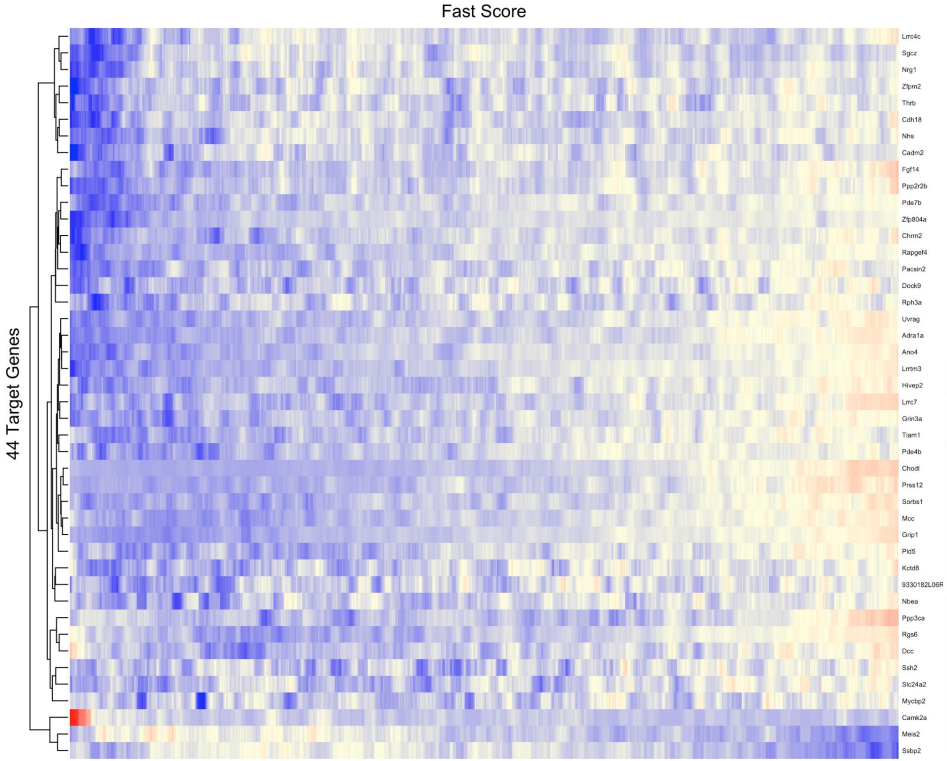
cuprous ion binding (GO:1903136)

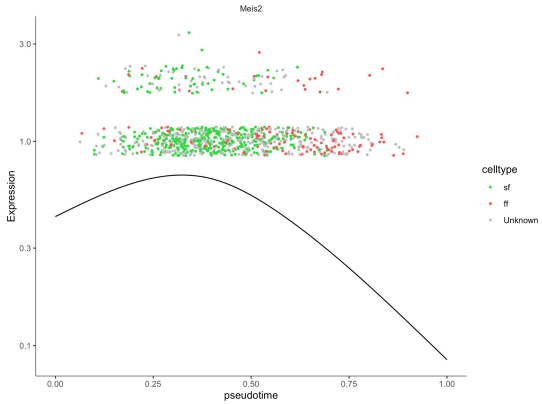
ionotropic glutamate receptor binding (GO:0035255)

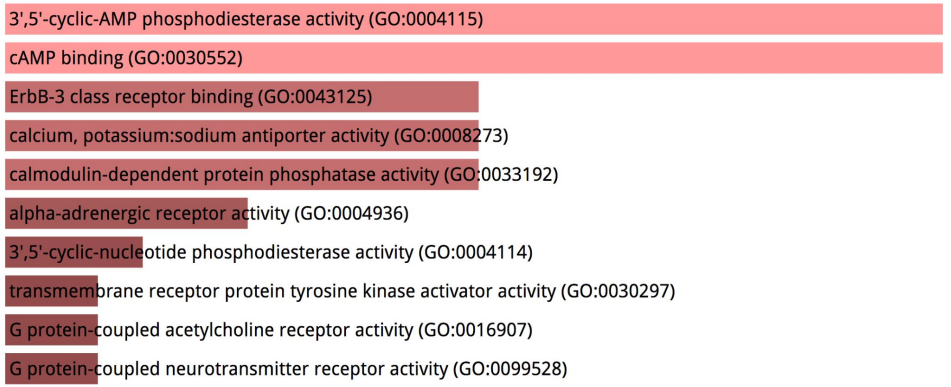
aldo-keto reductase (NADP) activity (GO:0004033)

phospholipase inhibitor activity (GO:0004859)

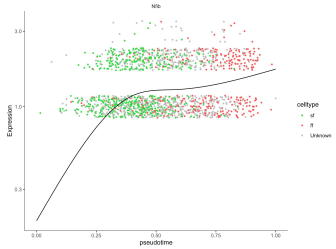
Meis2 - repressor of fast identity

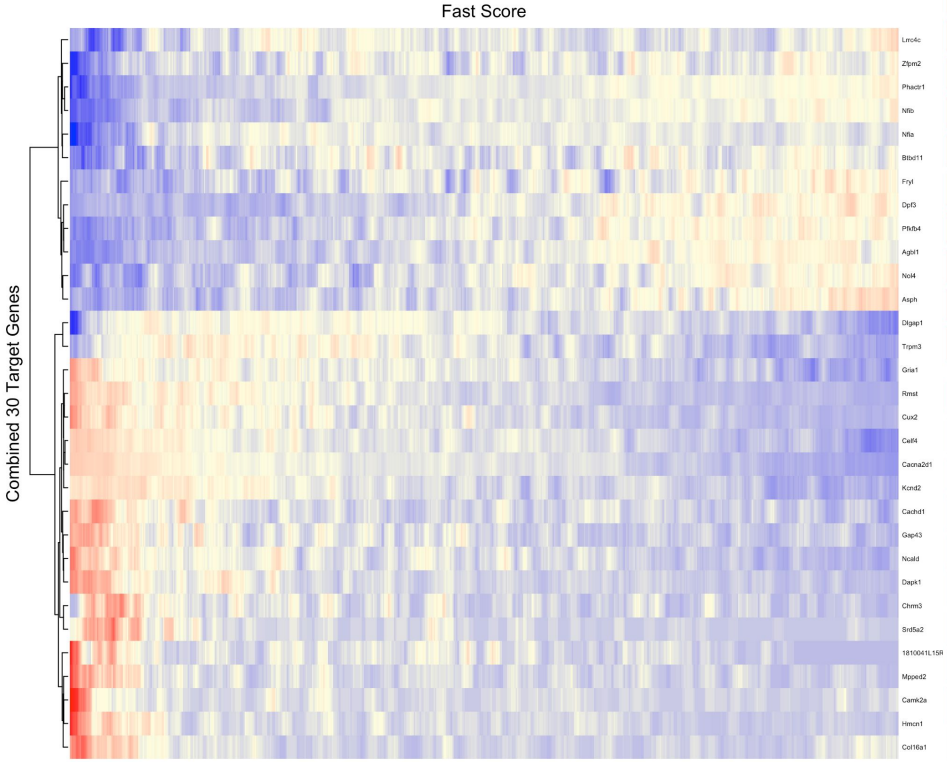






Nfib - activator of fast identity, repressor of slow identity





testosterone dehydrogenase [NAD(P)] activity (GO:0030283)

voltage-gated calcium channel activity involved in cardiac muscle cell action potential (GO:0086007)

G protein-coupled acetylcholine receptor activity (GO:0016907)

G protein-coupled neurotransmitter receptor activity (GO:0099528)

calmodulin-dependent protein kinase activity (GO:0004683)

calcium-dependent protein kinase activity (GO:0010857)

calcium-dependent protein serine/threonine kinase activity (GO:0009931)

voltage-gated calcium channel activity (GO:0005245)

outward rectifier potassium channel activity (GO:0015271)

AMP binding (GO:0016208)

fructose-2,6-bisphosphate 2-phosphatase activity (GO:0004331)

phosphofructokinase activity (GO:0008443)

sugar-phosphatase activity (GO:0050308)

cell adhesion mediator activity (GO:0098631)

metallocarboxypeptidase activity (GO:0004181)

carboxypeptidase activity (GO:0004180)

cell-cell adhesion mediator activity (GO:0098632)

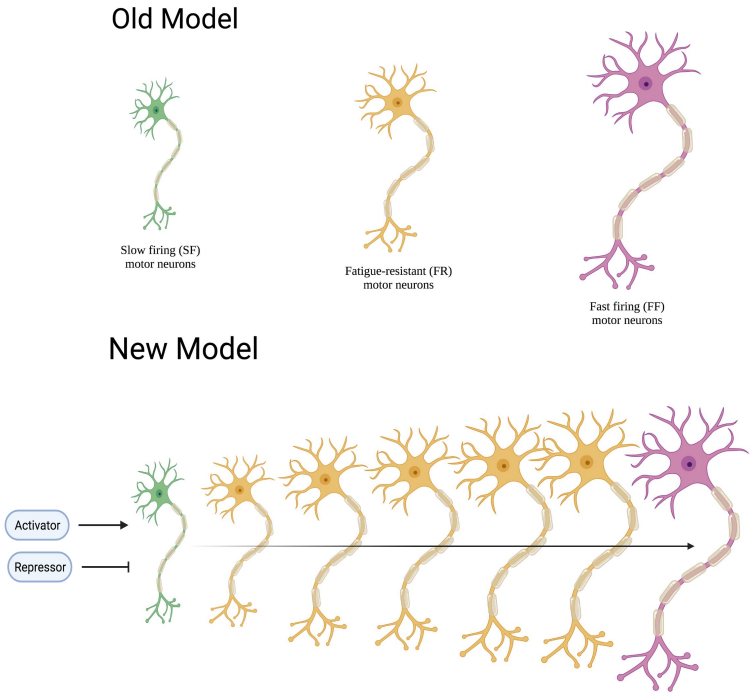
inorganic cation transmembrane transporter activity (GO:0022890)

metalloexopeptidase activity (GO:0008235)

ion channel activity (GO:0005216)

Discussion

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



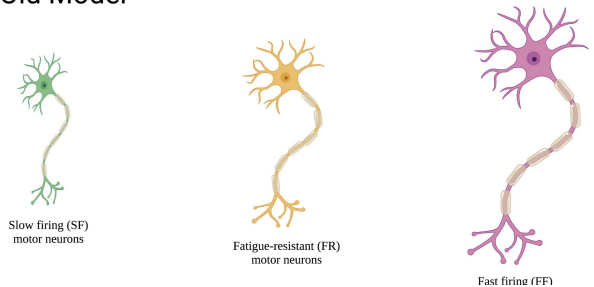
Thanks

- Jacob Blum, PhD
- Aaron Gitler, PhD
- Gitler Lab at Stanford

References

1. Blum, J. A. *et al.* Single-cell transcriptomic analysis of the adult mouse spinal cord reveals molecular diversity of autonomic and skeletal motor neurons. *Nat. Neurosci.* **24**, 572–583 (2021).
2. Taylor, J. P., Brown, R. H. & Cleveland, D. W. Decoding ALS: from genes to mechanism. *Nature* **539**, 197–206 (2016).
3. Kanning, K. C., Kaplan, A. & Henderson, C. E. Motor neuron diversity in development and disease. *Annu. Rev. Neurosci.* **33**, 409–440 (2010).
4. Hao, Y. *et al.* Integrated analysis of multimodal single-cell data. *Cell* **184**, 3573–3587.e29 (2021).
5. Cao, J. *et al.* The single-cell transcriptional landscape of mammalian organogenesis. *Nature* **566**, 496–502 (2019).

Old Model



New Model