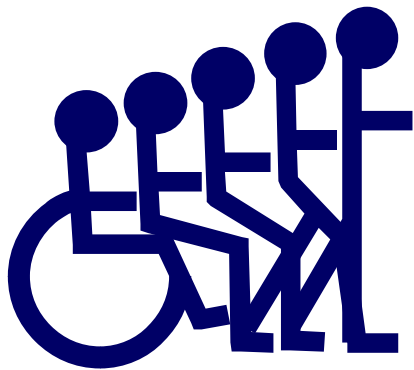


Using HCA to Identify a Transcription Factor Family that Regulates the Intrinsic Ability of Neurons to Extend Axons

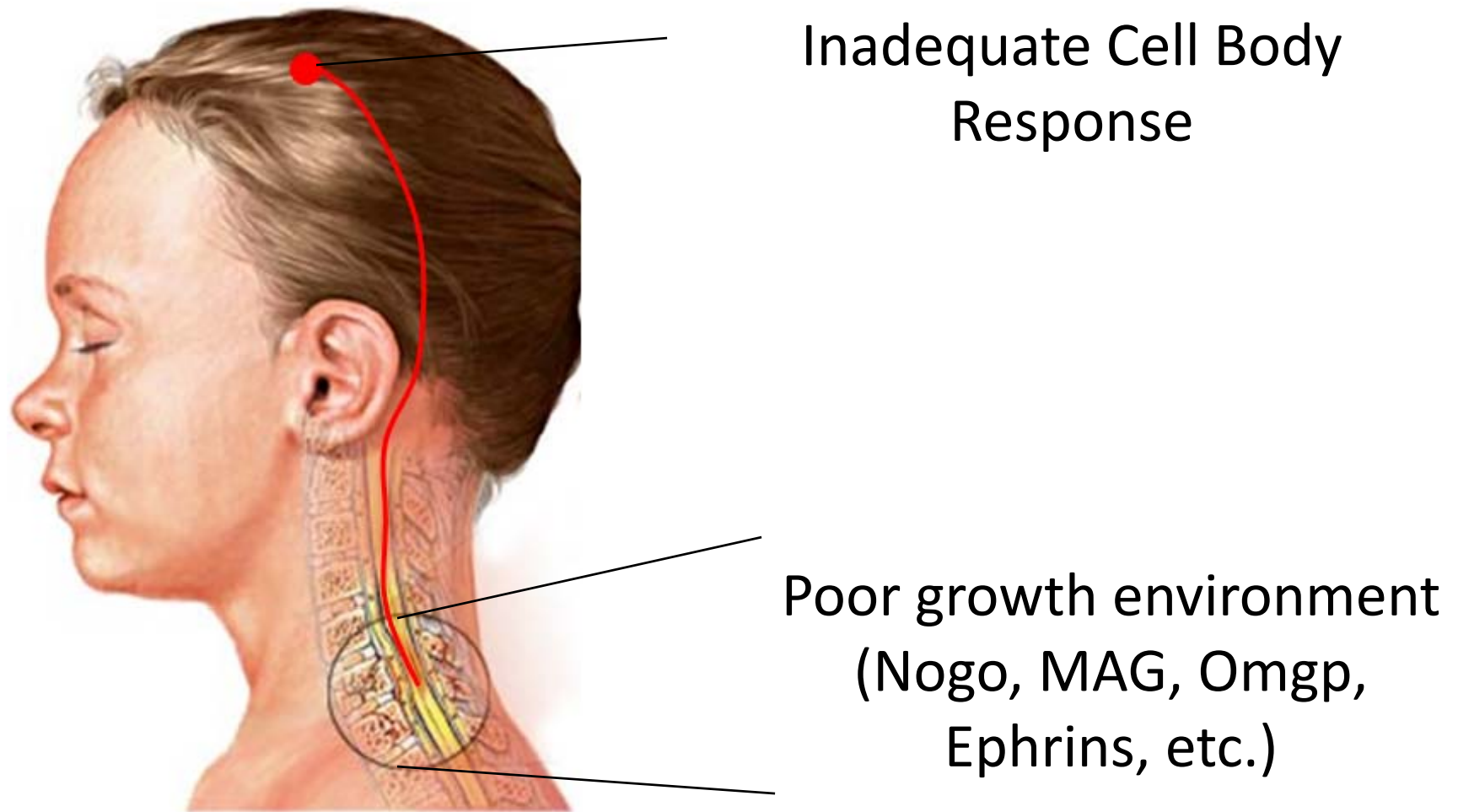


Murray Blackmore,
Vance Lemmon, Darcie Moore,
Jeff Goldberg & John Bixby
Miami Project to Cure Paralysis
University of Miami Miller School of Medicine

UNIVERSITY OF MIAMI
MILLER SCHOOL
of MEDICINE

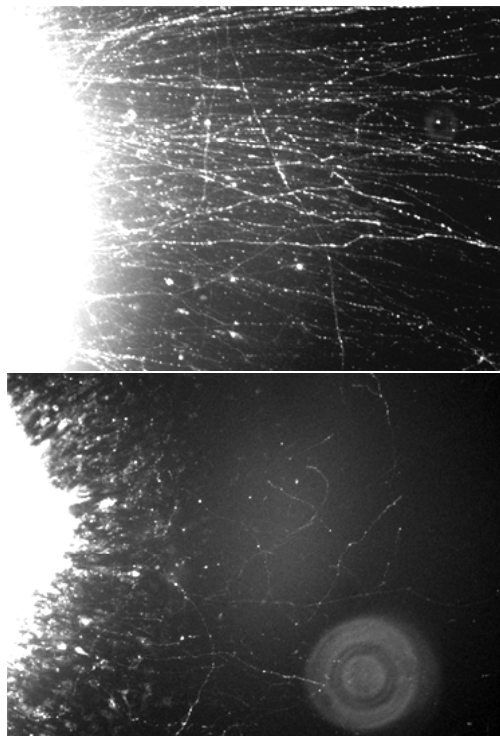


Why can't axons in the central nervous system (CNS) regenerate?



Young neurons regenerate better than old

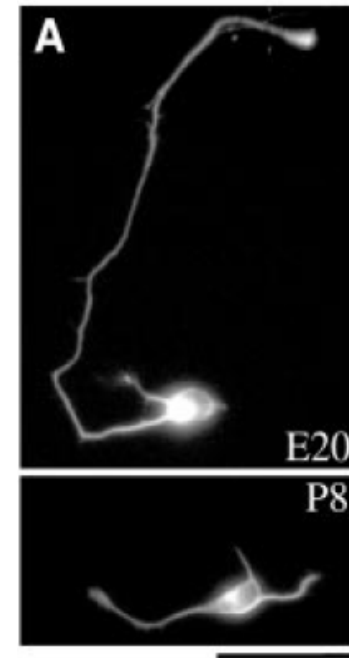
brainstem



E9

E15

retinal ganglion cell



A

E20

P8

Research Strategy

- 1) Identify differentially expressed genes
- 2) Unbiased, phenotypic screening in neurons

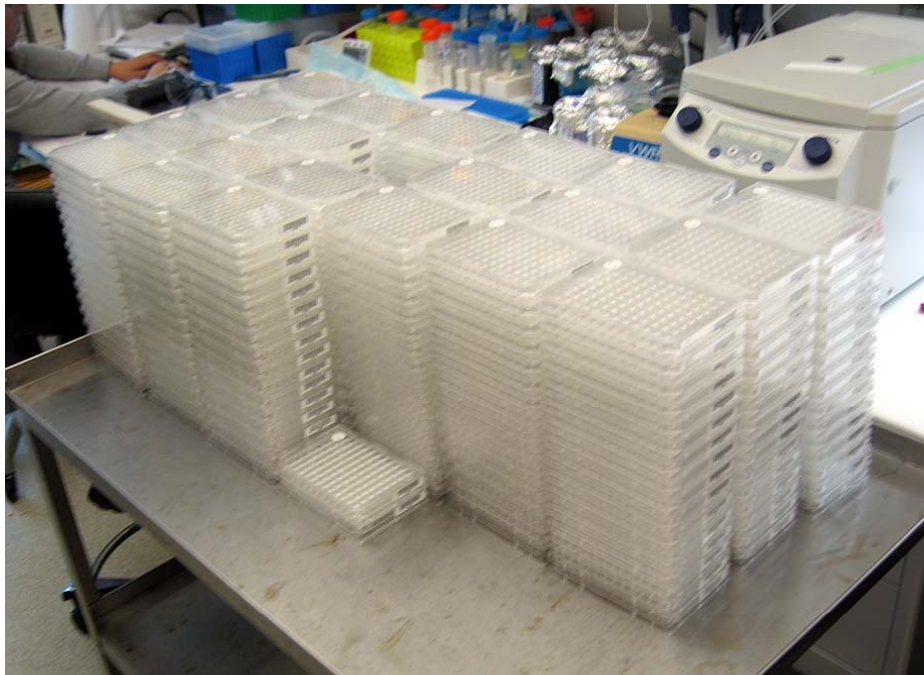
-Older neurons might upregulate inhibitory genes, or downregulate stimulatory genes

Identifying a list of genes...

- CST regeneration ability is lost between P3 and P16
- Microarray analysis of CST neurons at E18 and at P14 (Arlotta, P. et al., Neuron 45: 207, 2005)
- picked 237 genes that decreased and 834 genes that increased (total 1071)

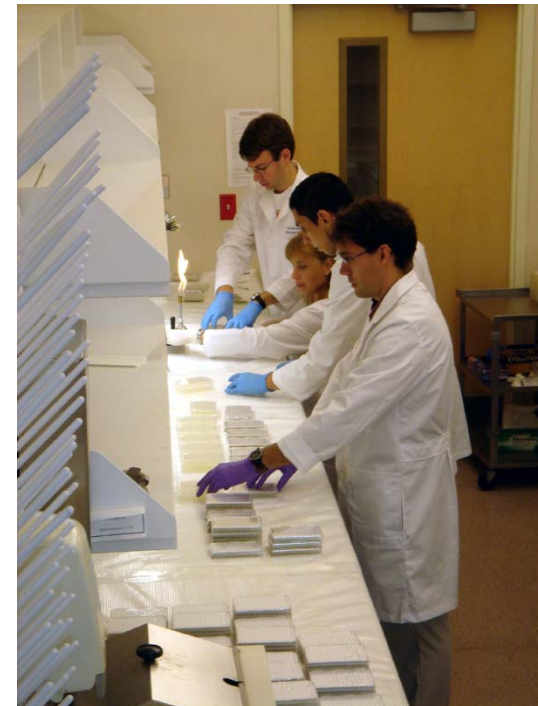
Where do we get all those cDNAs?

Open Biosystems Library

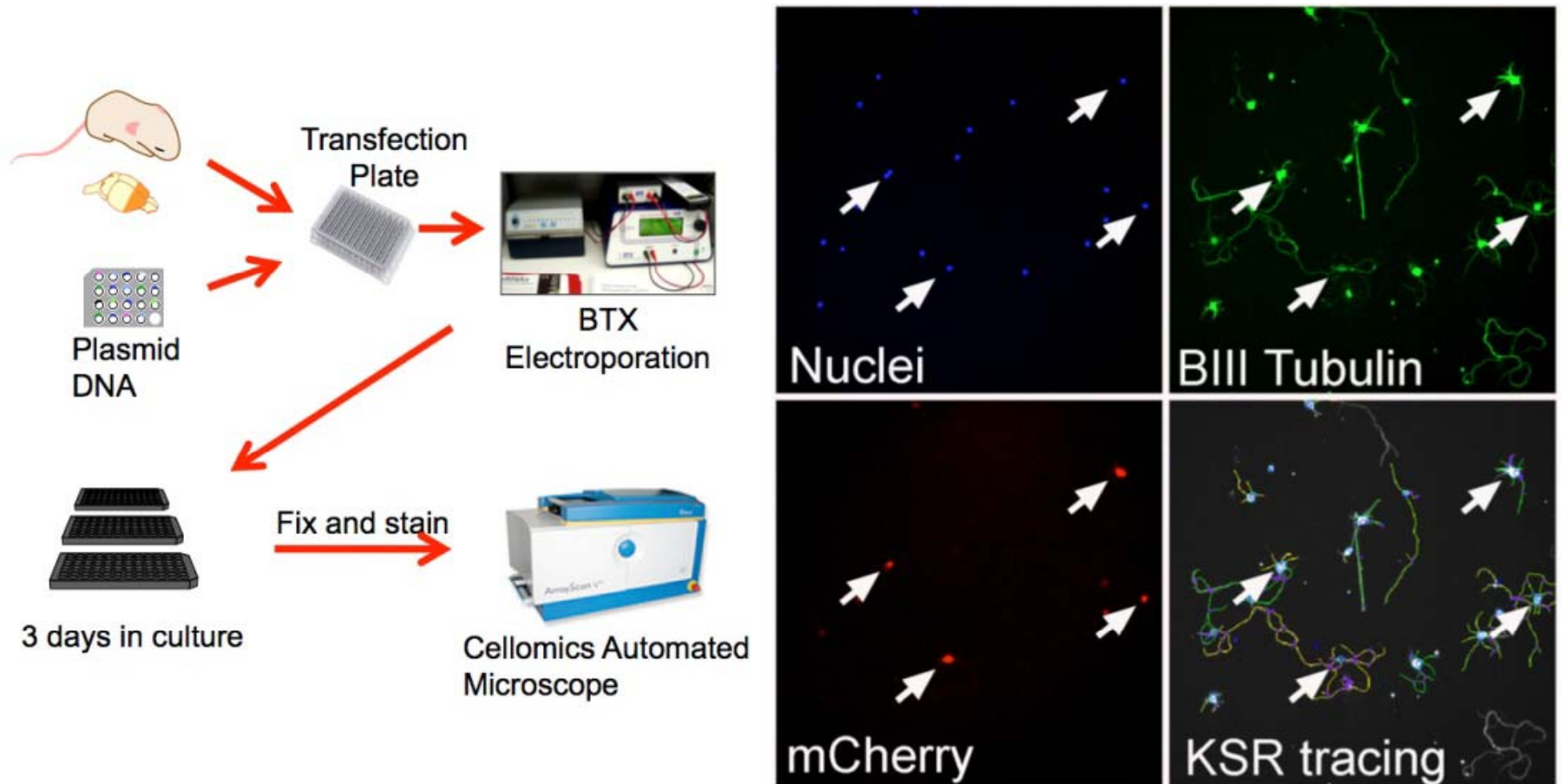


743 clones; 445 genes

6,200 human genes
9,800 mouse genes
About 160 96-well plates

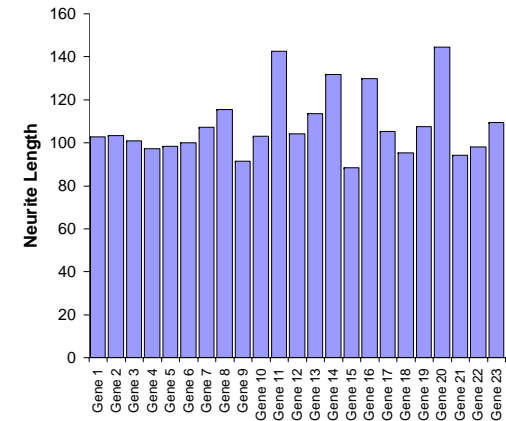
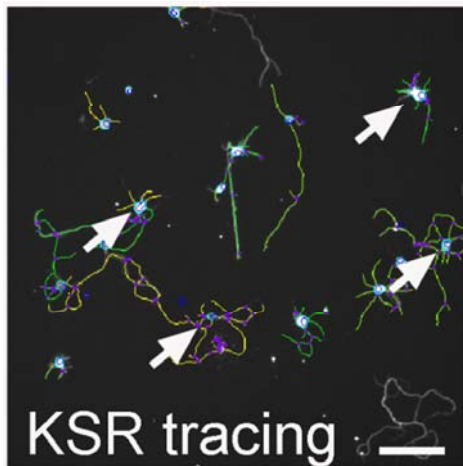


Workflow of screen



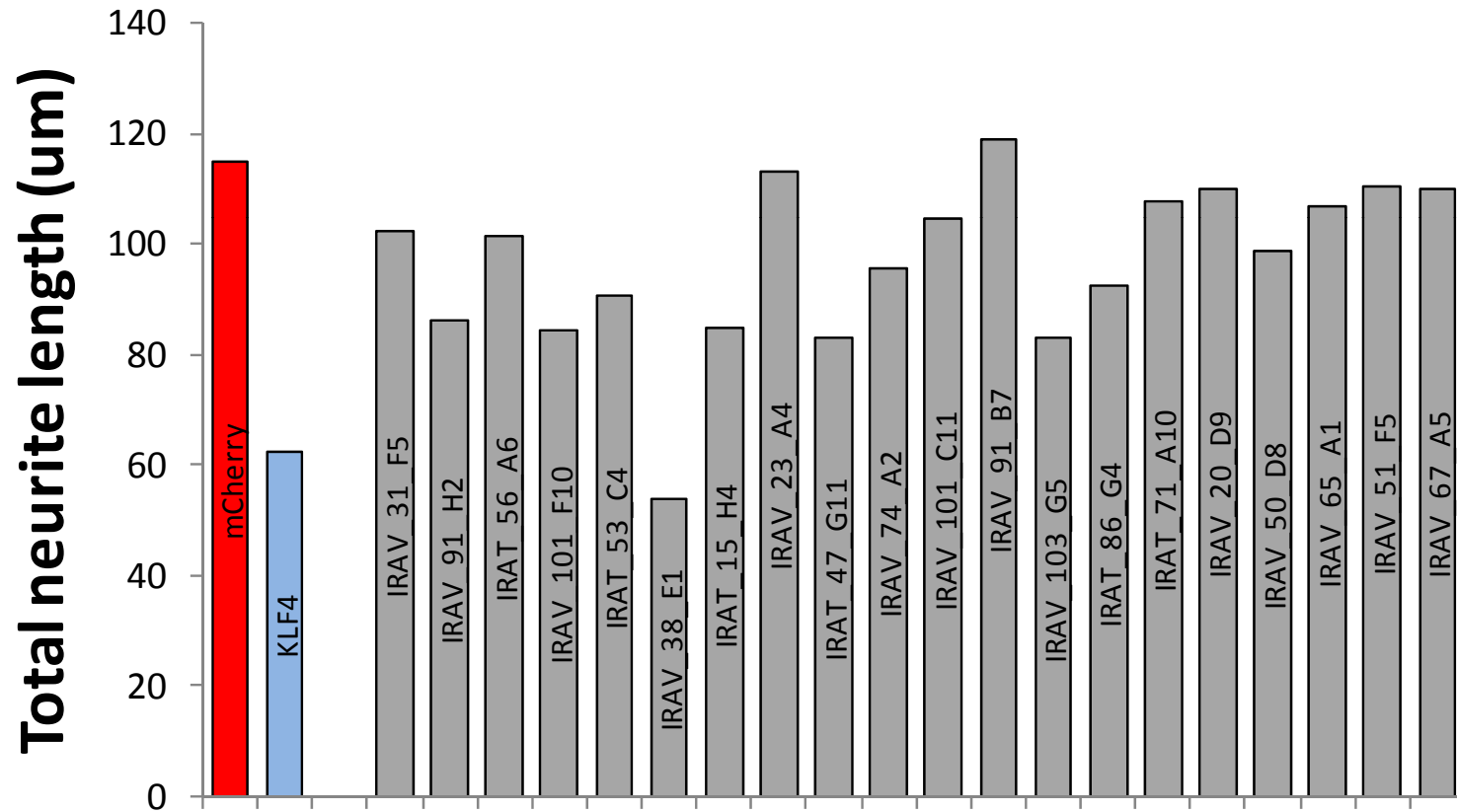
Semi-automated data analysis

> 100,000 cells per experiment

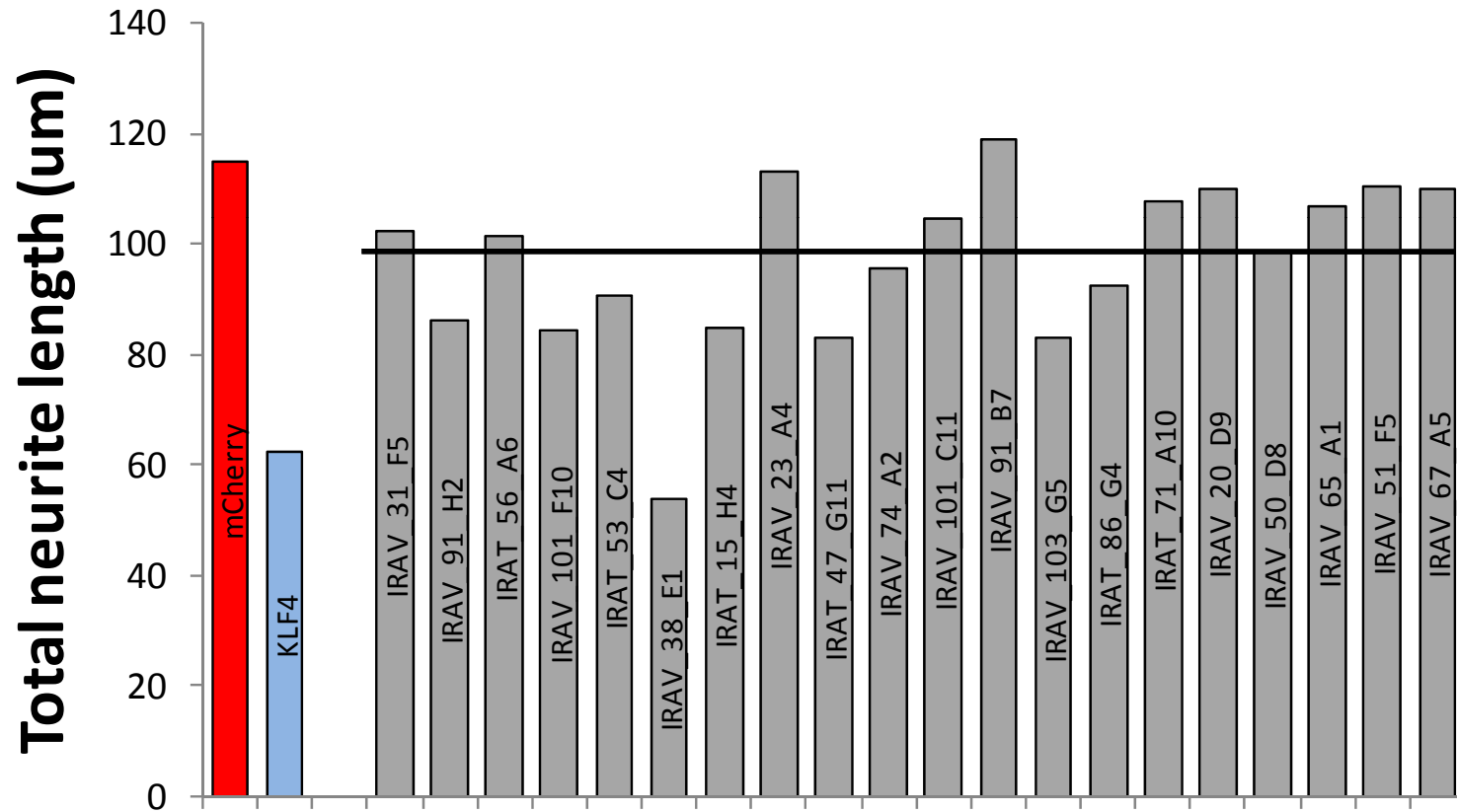


- Group data
- Identify transfected cells
- Identify artifacts

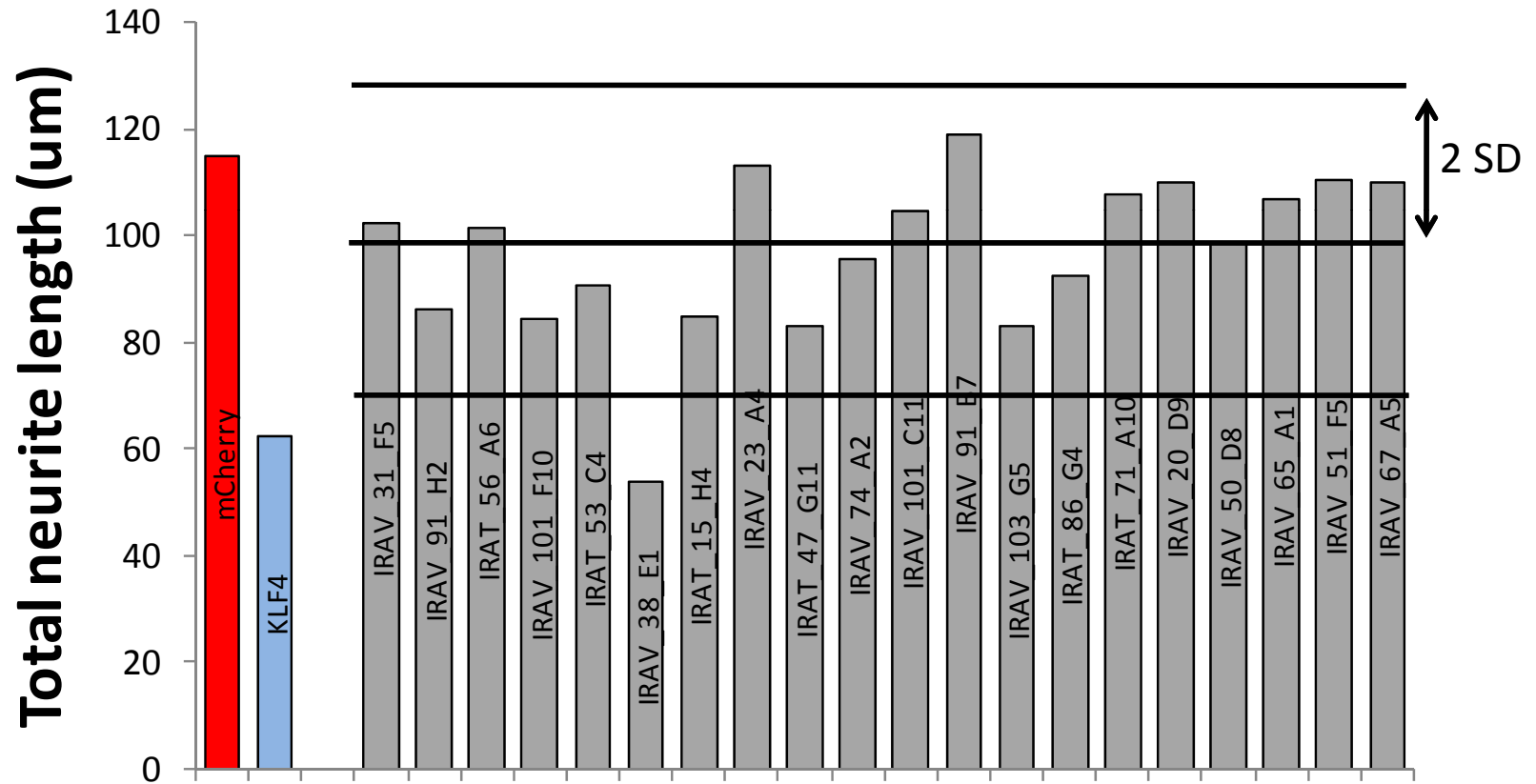
Data normalization



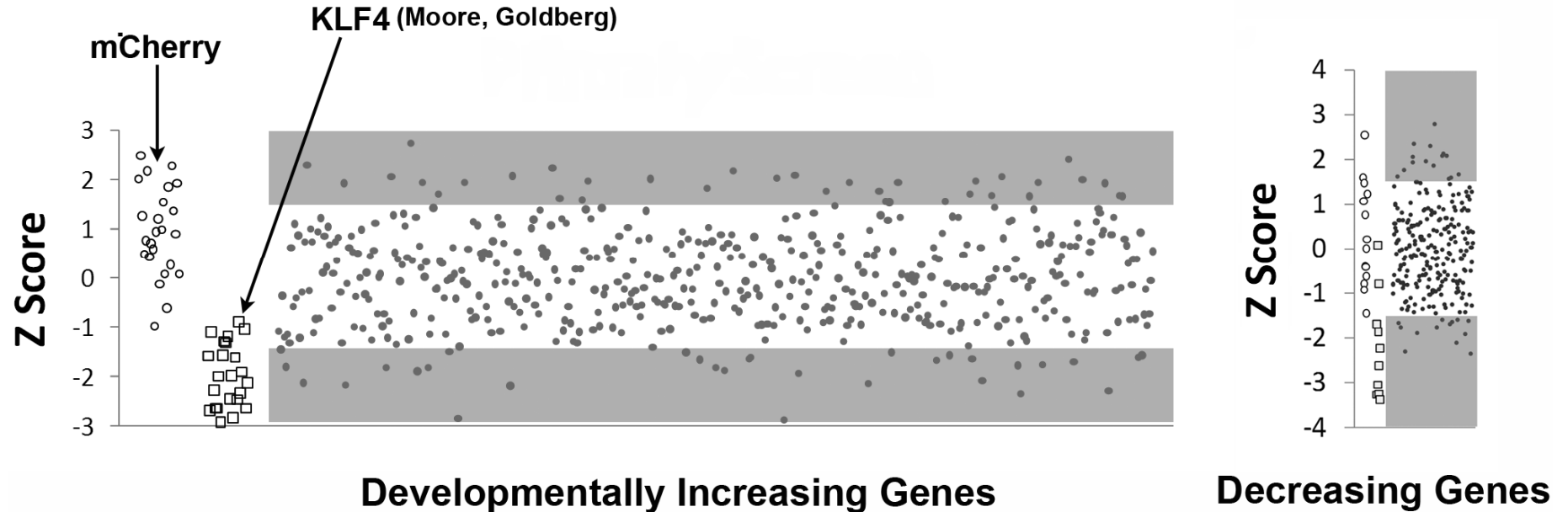
Data normalization



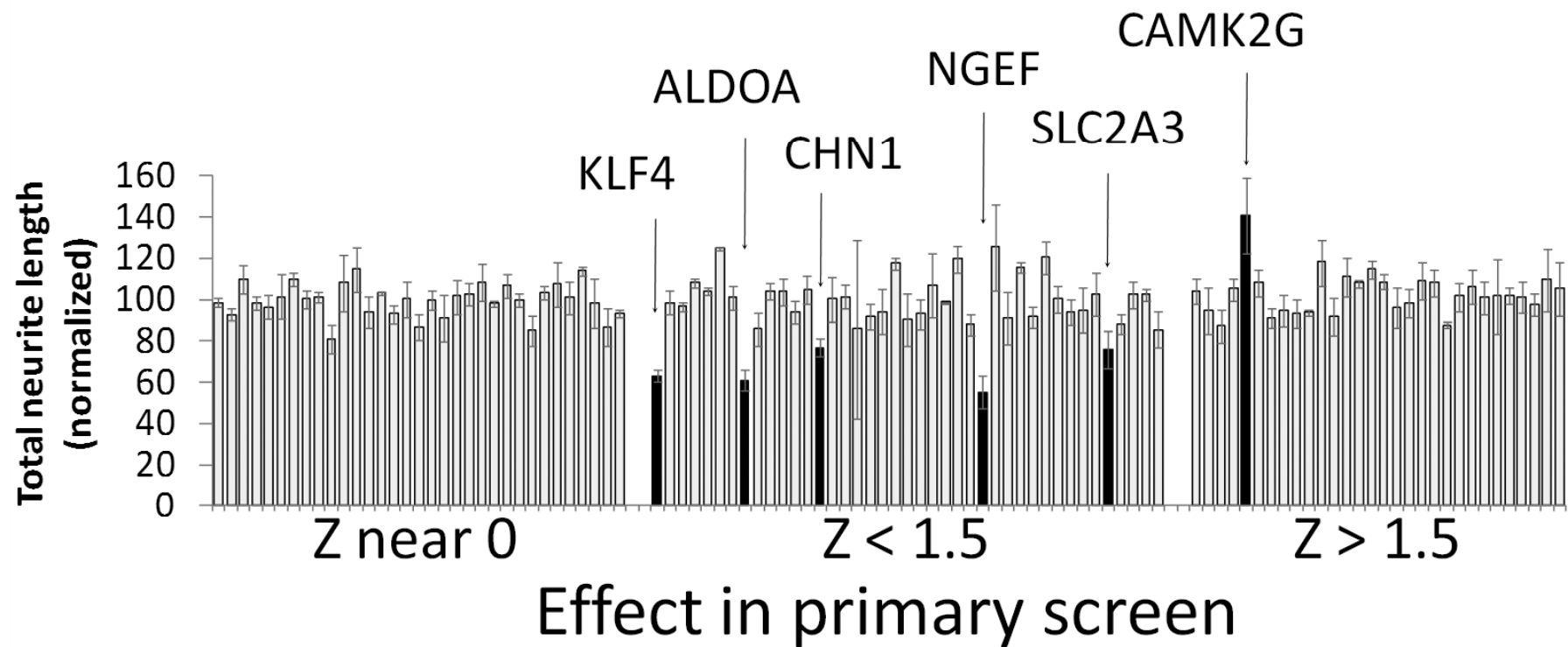
Data normalization



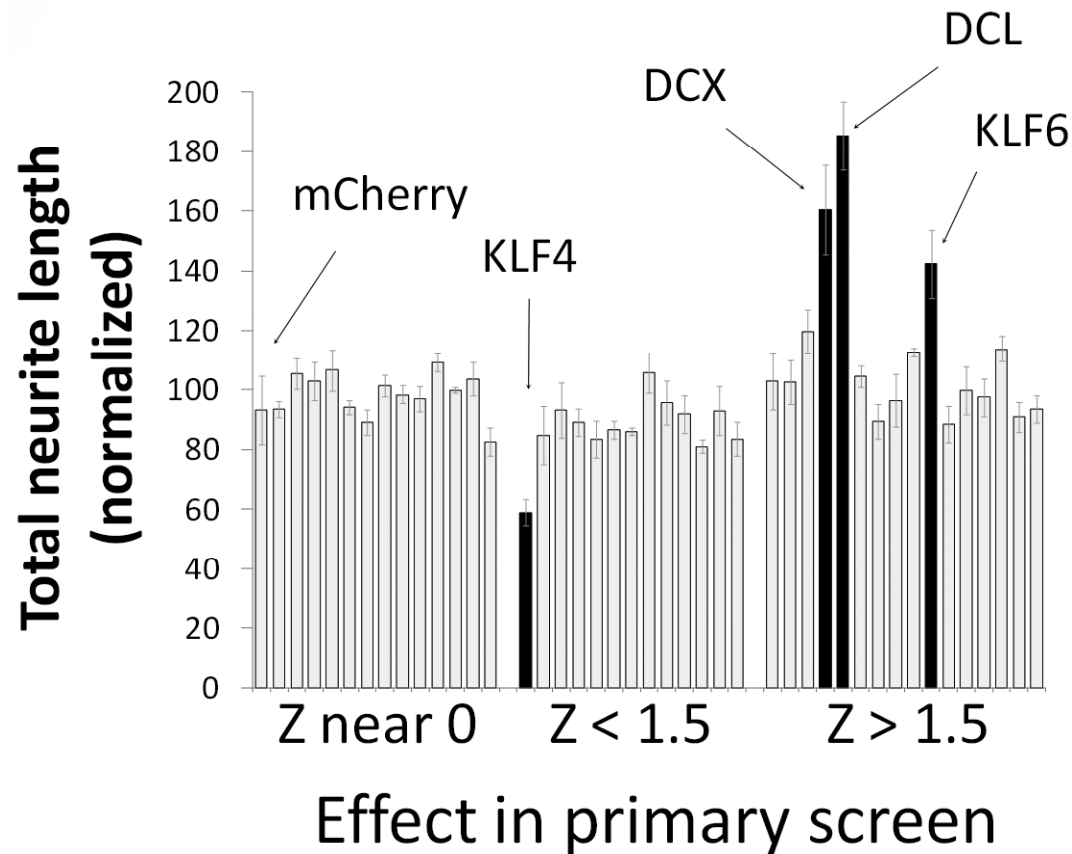
Primary screen of developmentally changing genes identifies candidates that alter neurite growth



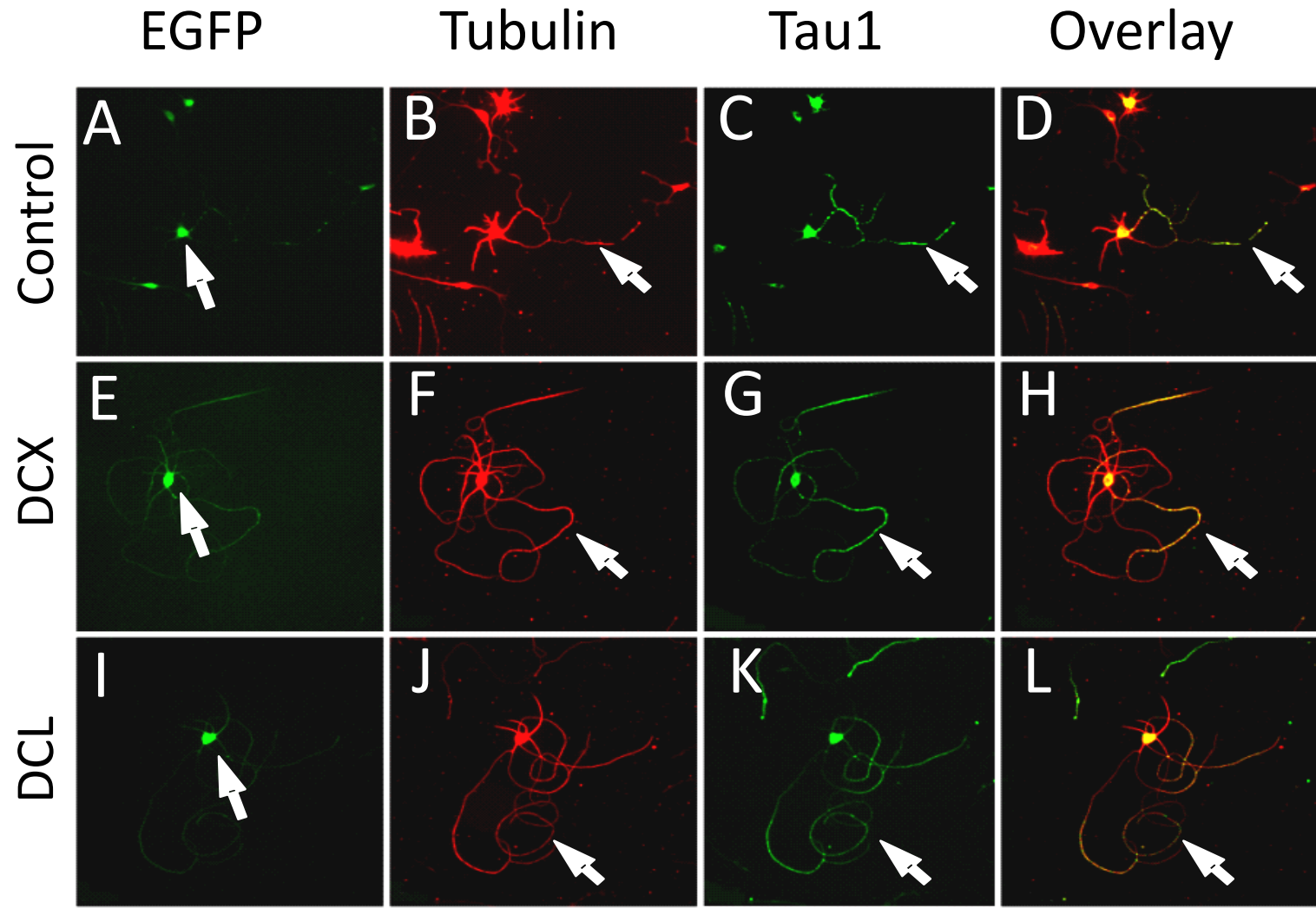
Developmentally increasing genes inhibit neurite growth



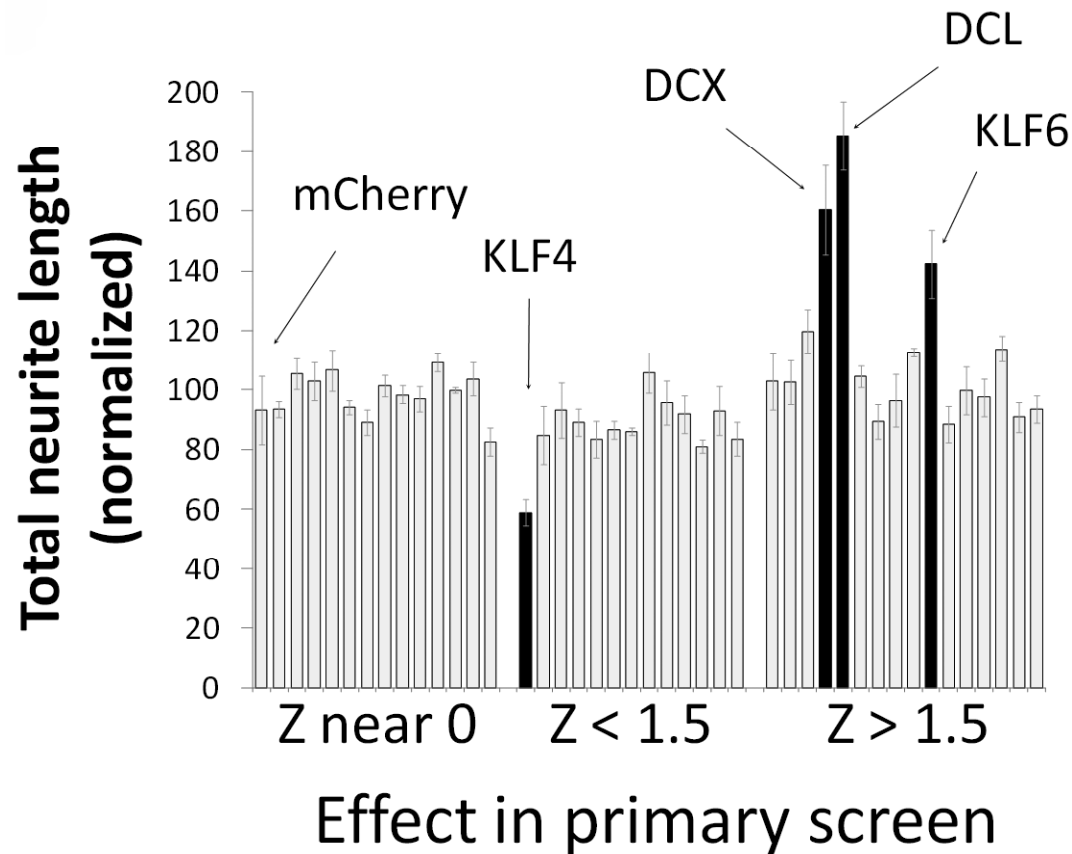
Developmentally decreasing genes augment neurite growth



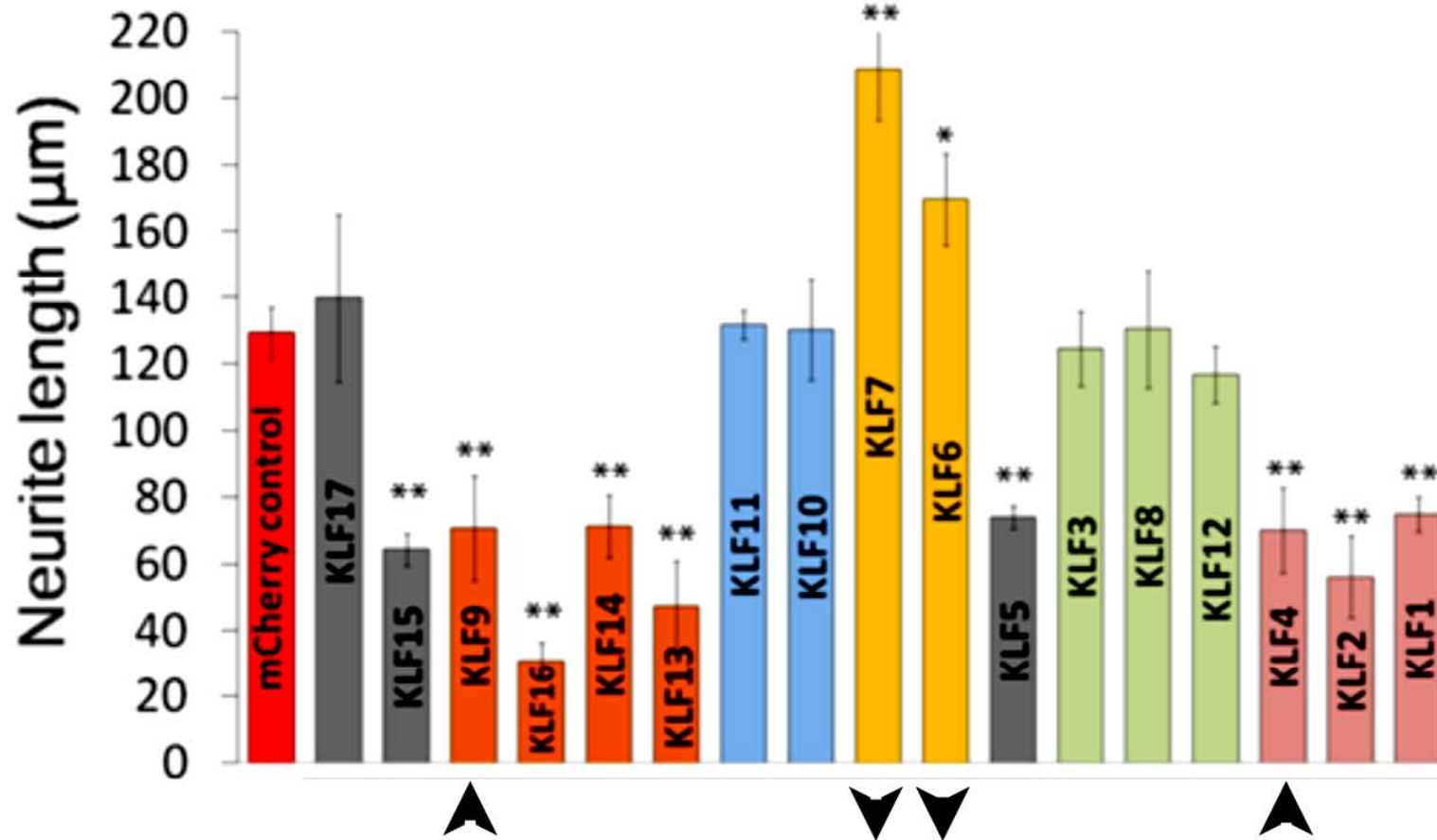
Doublecortin increases axon length in postnatal cortical neurons



Developmentally decreasing genes augment neurite growth

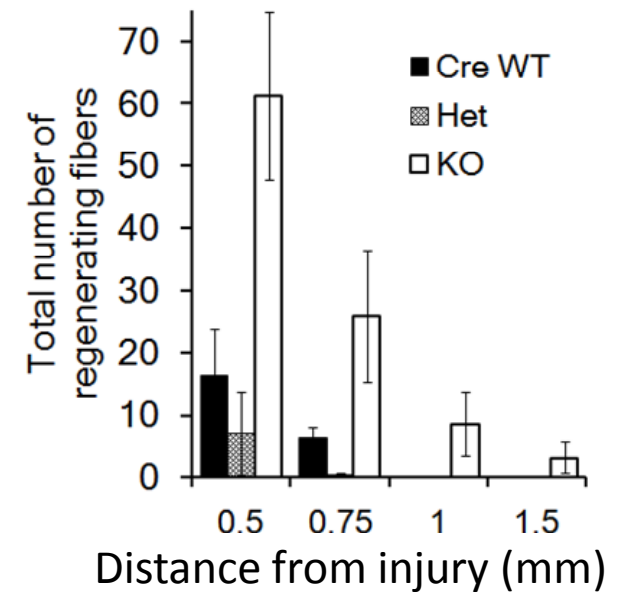
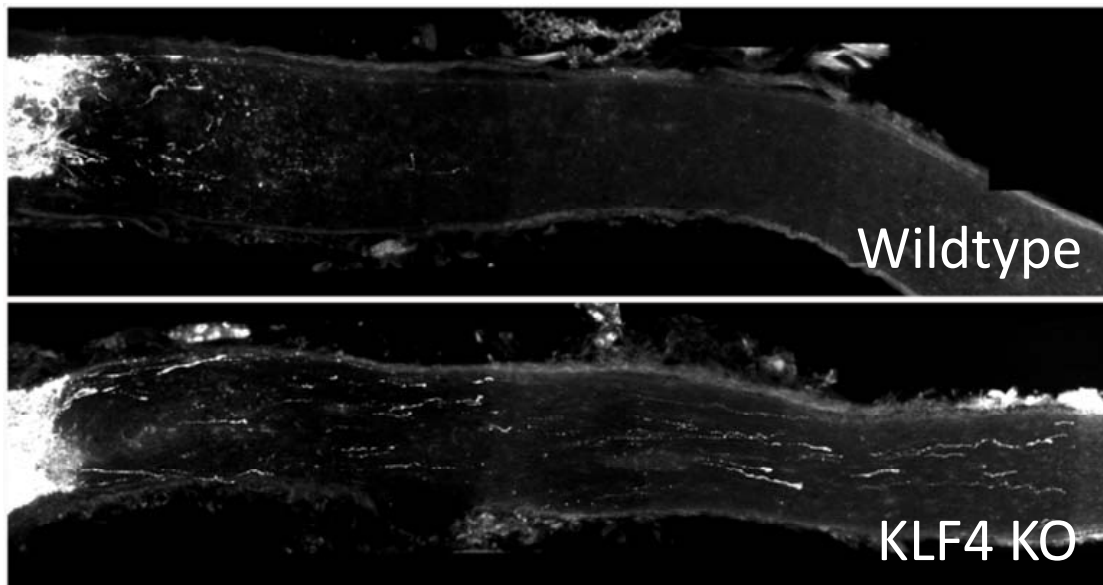


Multiple KLFs regulate axon growth



Moore, Blackmore et al. (2009). Science, 326: 298-301.

Transgenic knockdown of one growth-inhibitory KLF enhances axon regeneration *in vivo*



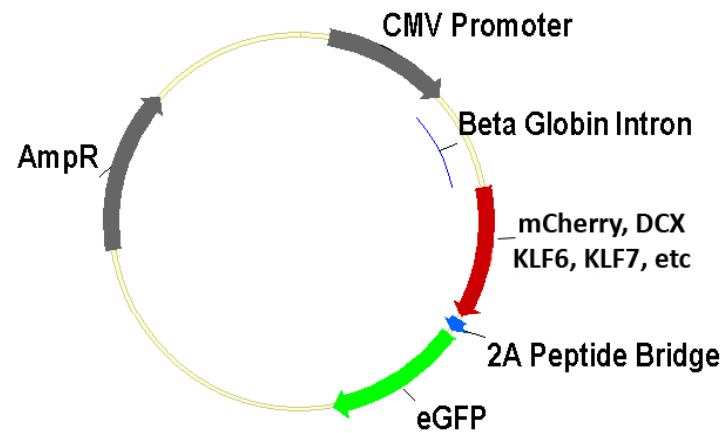
What are the relevant KLF target genes?

- Overexpress inhibitory, stimulatory, or neutral KLFs in primary CNS neurons
- Microarray analysis
- Screen transcriptional targets in neurite outgrowth assays

Gene Therapy for Rats

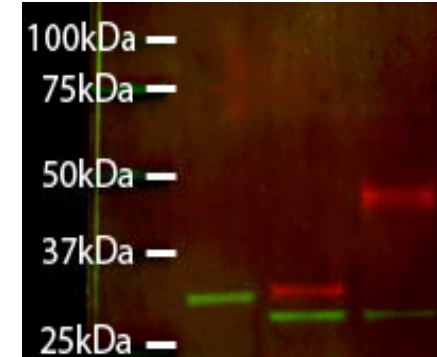
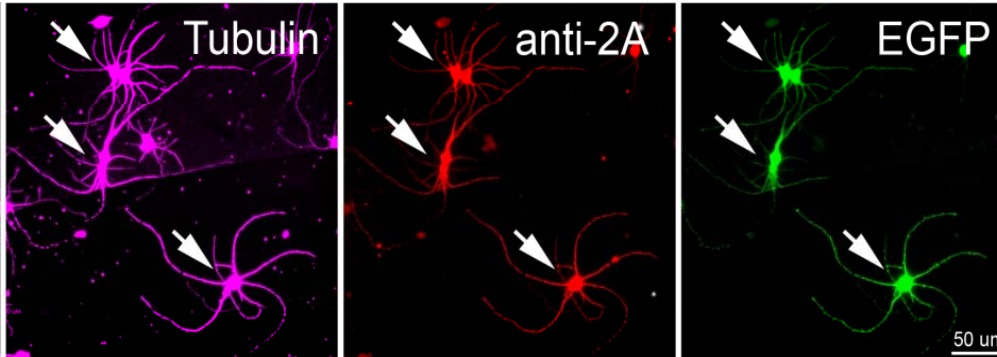
- Overexpress growth promoting genes
- Knock down growth-suppressive genes
- Overexpress/knockdown in combination

A 2A peptide strategy to overexpress multiple proteins

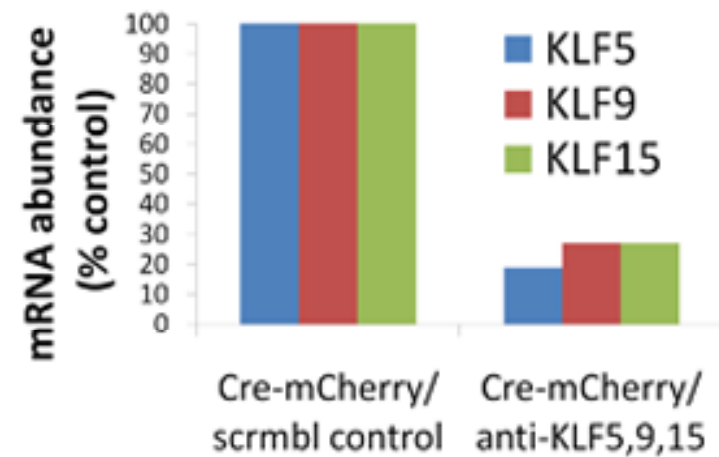
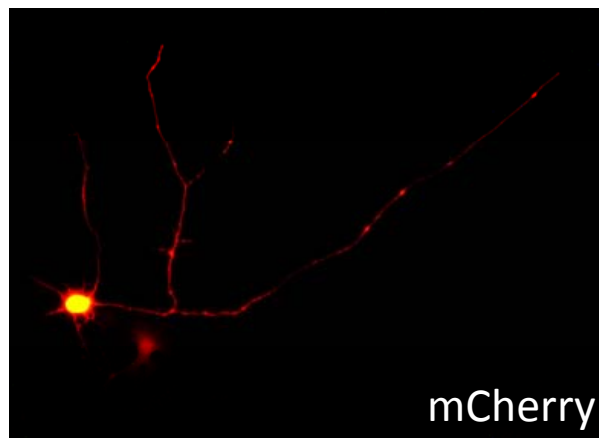
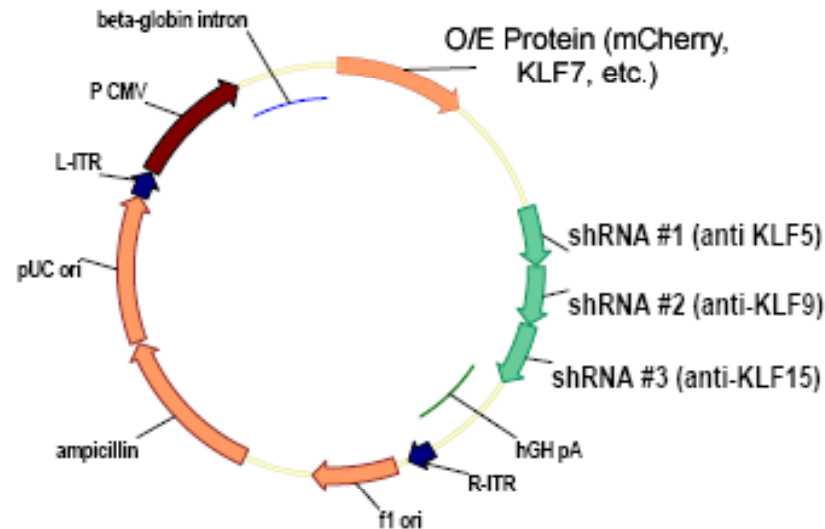


mCherry-2A-EGFP
mCherry-2A-DCX

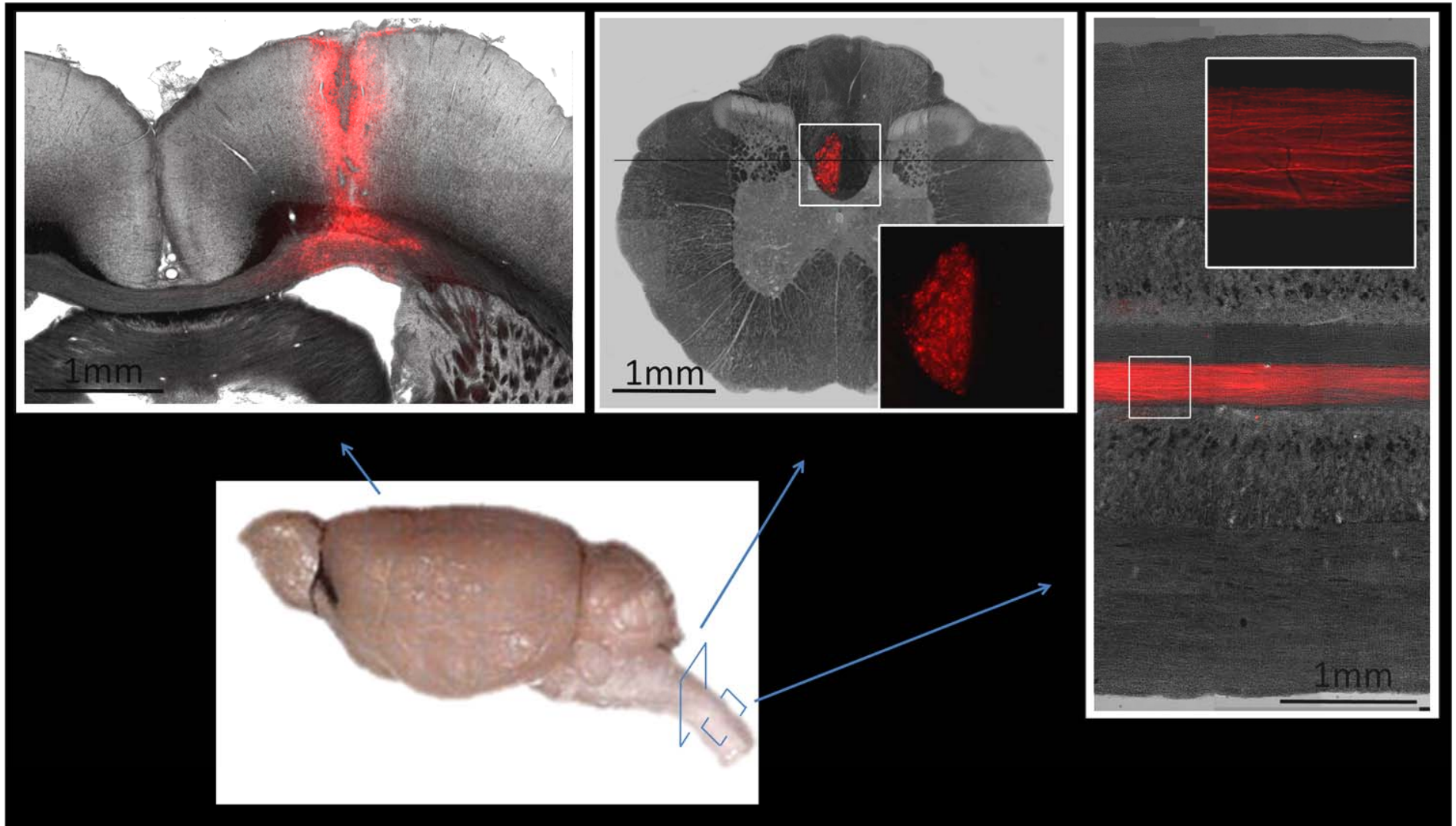
Doublecortin-2A-EGFP



A concatenated shRNA strategy to knock down multiple proteins



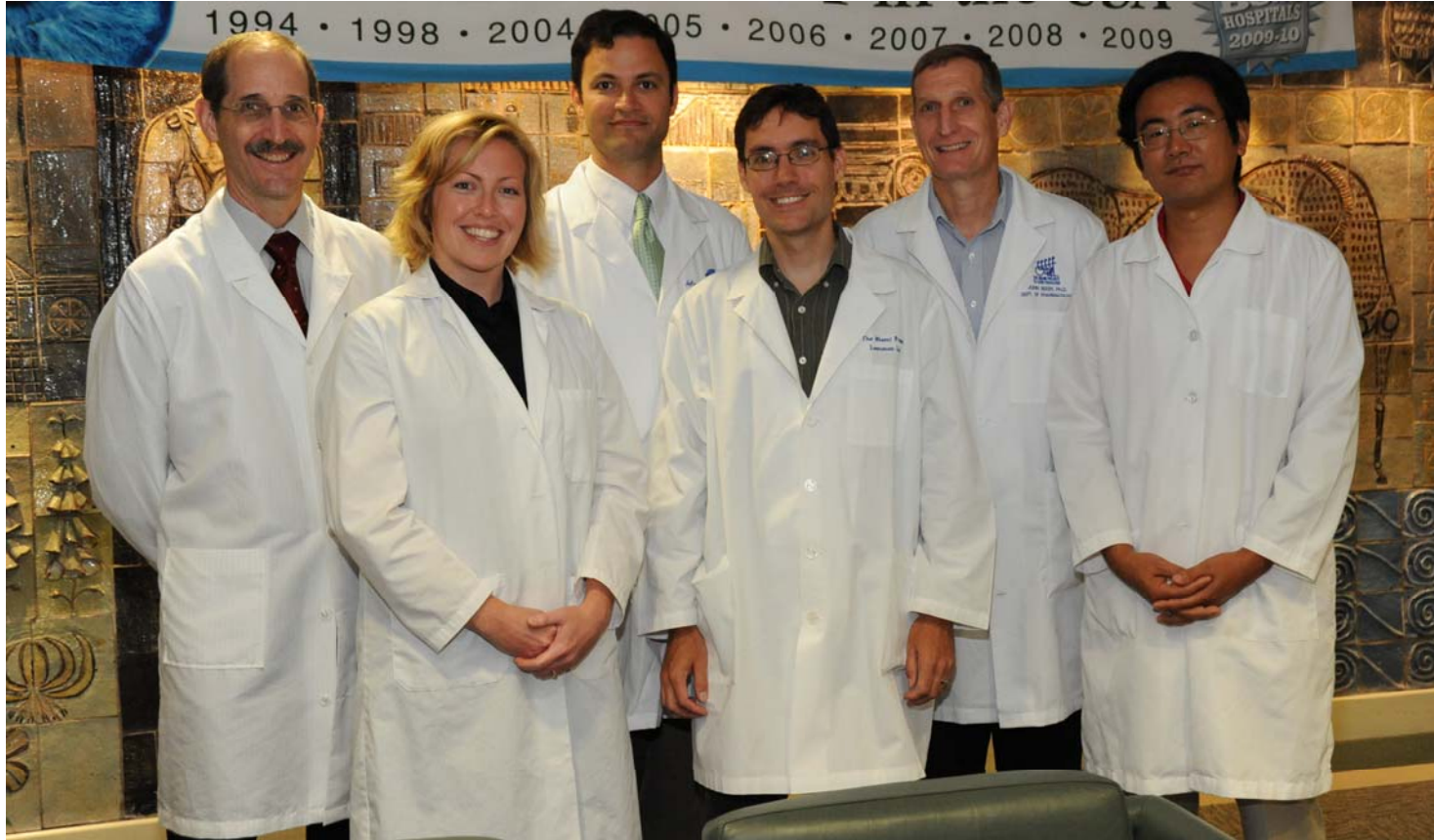
Lentiviral vectors allow transduction and tracing of corticospinal axons



Summary

- Screened >400 developmentally regulated genes in postnatal cortical neurons
- Identified 4 growth suppressors and 4 growth enhancers
- KLF transcription factors are top “hits”
- Developing tools to test genes in vivo in spinal cord injury

Acknowledgements



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