

Uncovering New Knowledge About Nerve Growth Using Clustering and Thresholding: Distinguishing Responders From Nonresponders



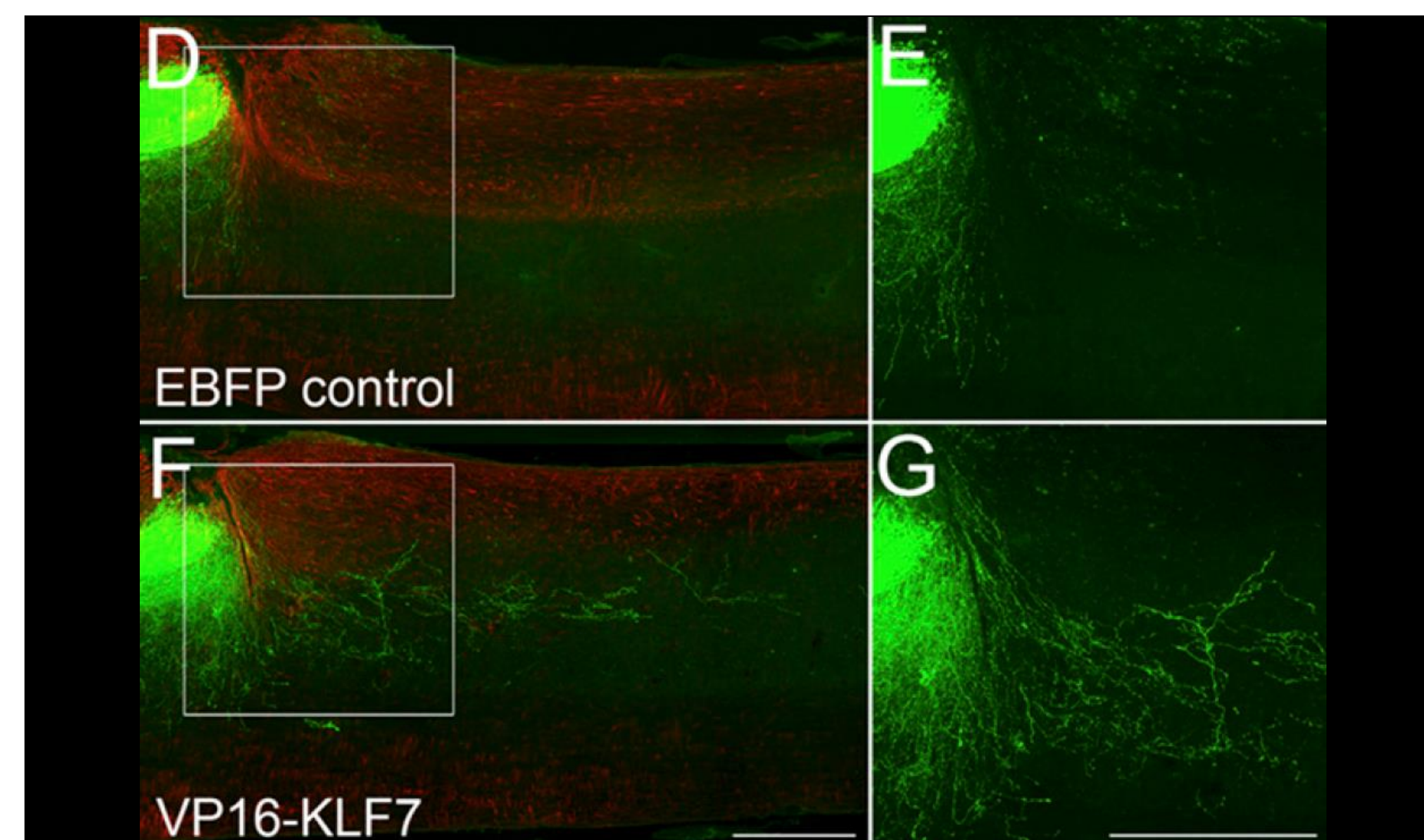
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Introduction

At a spinal cord injury site, the axons in the area are often severed. This disrupts the communication between the neurons in the brain and motor neurons. The axons do not grow back by themselves, so treatments are being developed to overcome the environmental and intrinsic inhibitions of growth the neurons face. In these experiments we are working with cortical neuron cells in vitro, so the treatments focus on activating the neurons' growth program, which is an issue concerning the intrinsic state of the neuron.

Treating Severed Nerve

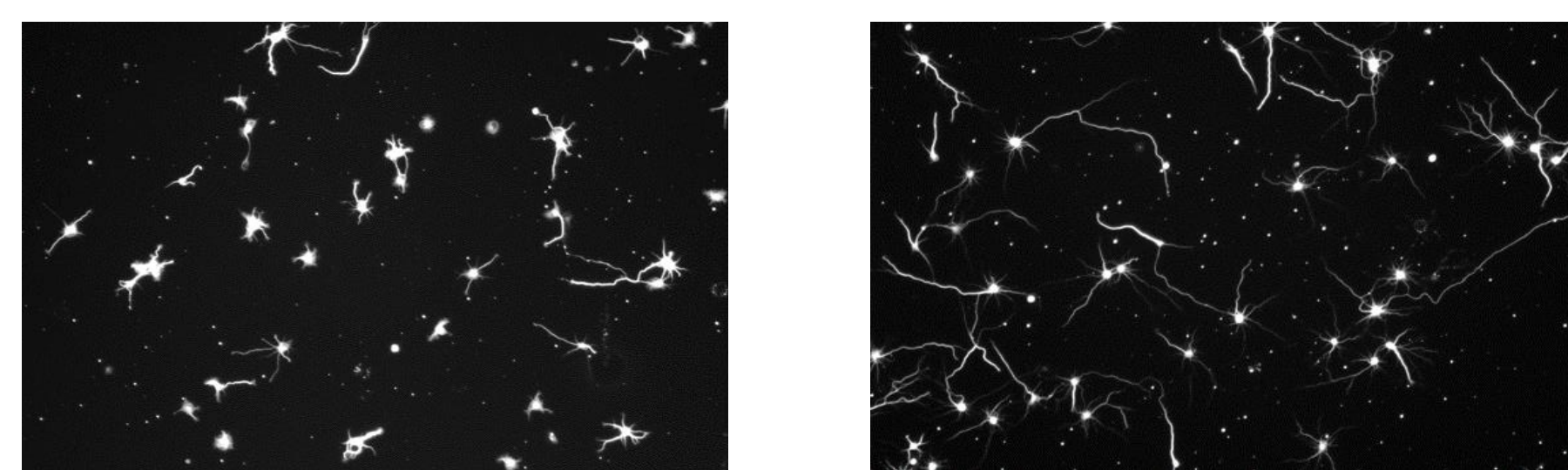


Corticospinal tract axons cut with a knife are able to grow long distances if treated with a transcription factor (F, G) but not in controls (D, E).

Transcription Factors

One of the treatment methods currently being researched is using viruses to transfect cells with different transcription factors, which alter gene expression. We look at many transcription factors, as well as combinations of them, and see how each treatment affects neurite growth in vitro.

Effective vs Ineffective Treatments

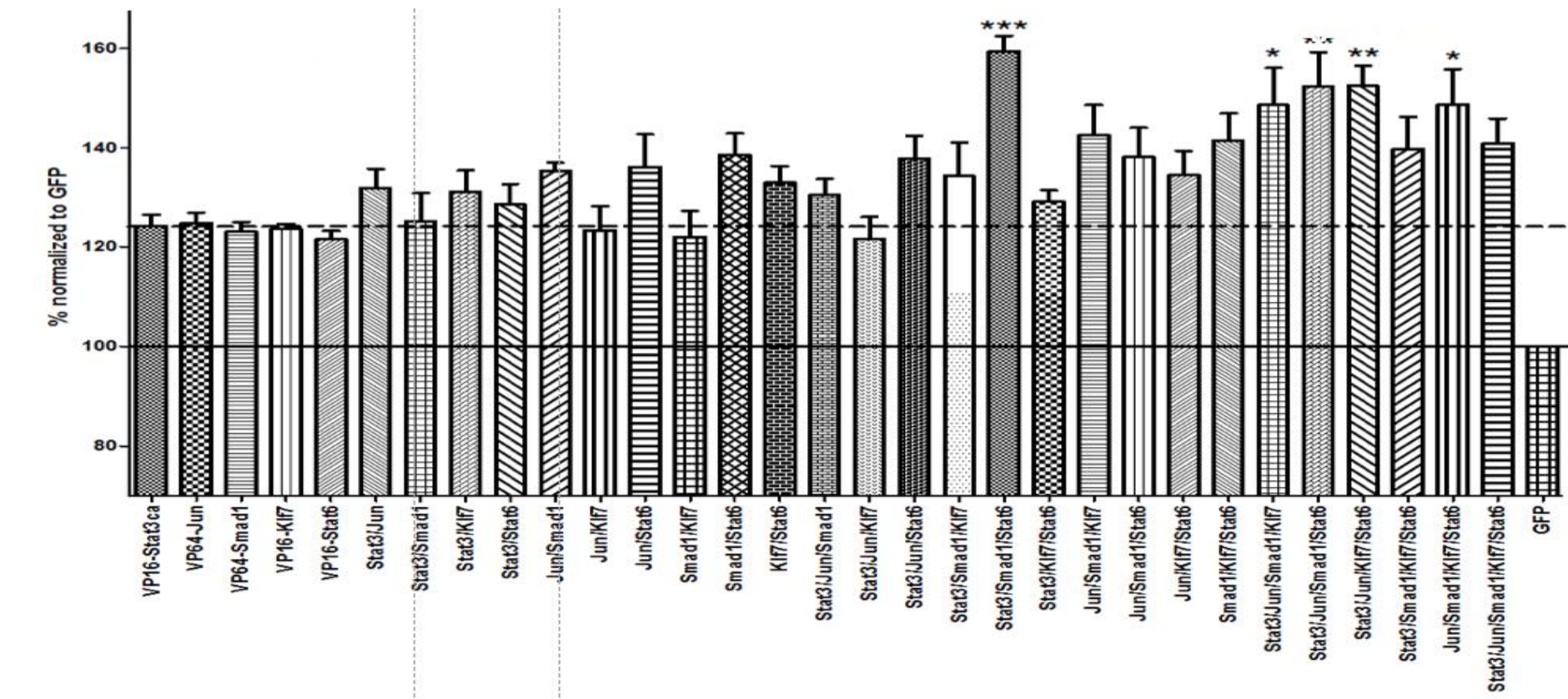


Effective treatments (e.g. image on right) alter neurite length, neurite branching and neurite numbers. Ineffective treatments (e.g. image on left) show little growth.

Previous Analysis

In the past, the analysis of data collected from these experiments has involved only a few of the many (around 30) features collected. This presents us with information that certain treatments do a better job than others at increasing the average values for some of the important cell features, but not much beyond that.

TF Average Neurite Growth Values



Average neurite growth by treatment group gathered from past experiments. This graph in particular provided the argument that combinations of treatments were more effective.

Mission

1. See if different treatments alter neurons' phenotypes in distinct ways through multidimensional analysis
2. Generally gather more information from the data than just the averages of a few features

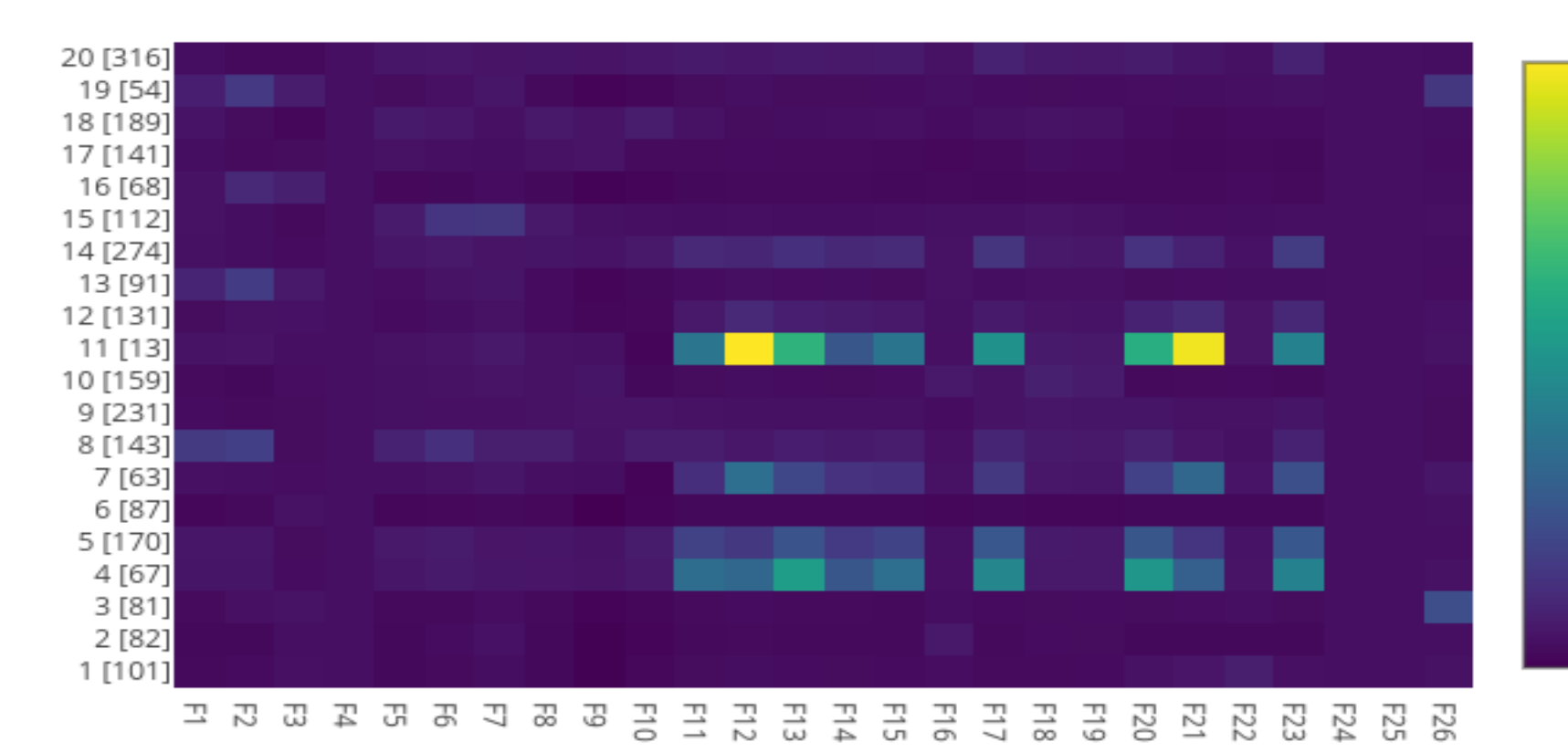
Data Collection / Normalization

Each experimental plate includes the different treatments that are being analyzed, as well as a control group, and a *no virus* group. The *no virus* group is used to filter out non-transfected cells in the other treatment groups. Then, we normalize each remaining point to the median feature values of the control group, where the new values are each point's *median absolute deviation* from the control, otherwise known as *robust z-score*.

Clustering Attempts

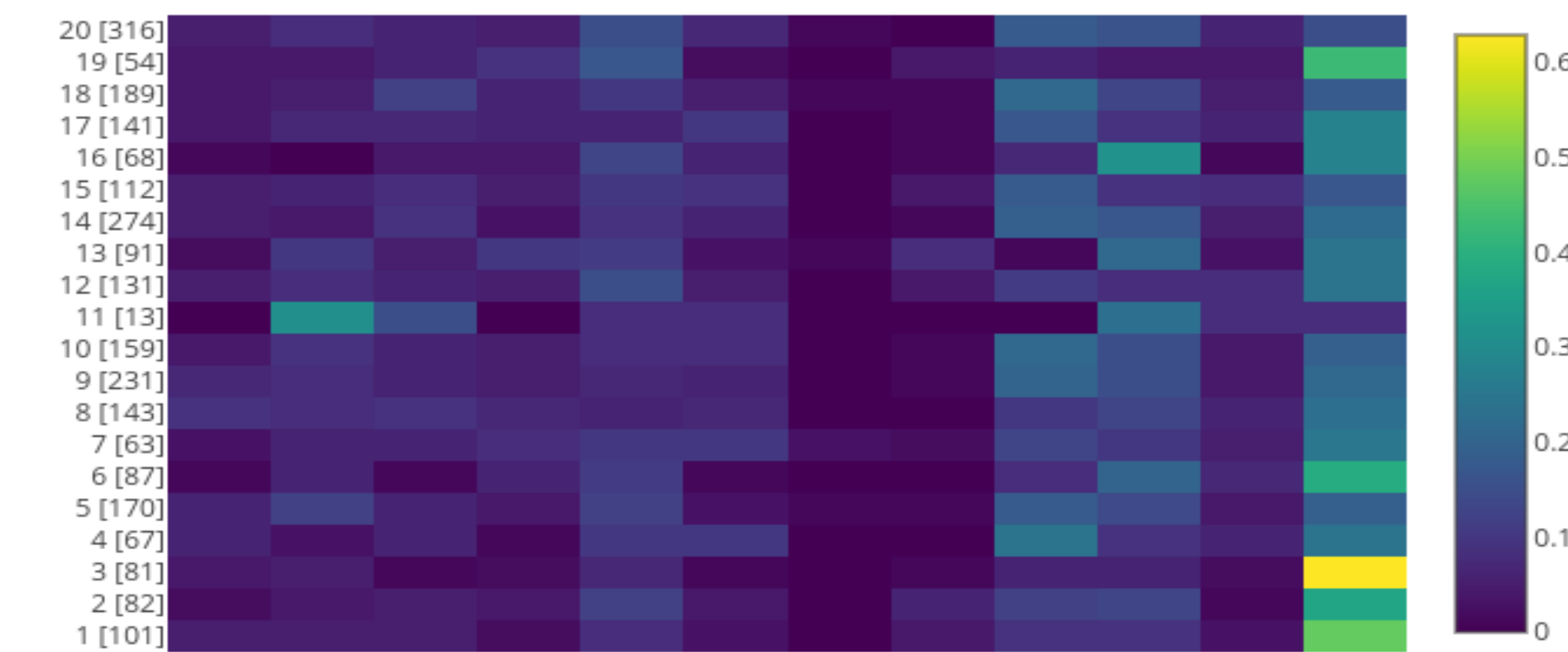
The goal with clustering was to see if any of the clusters are dominated by certain treatments, or at the very least, if the control group would be easily separated from the rest of the more effective treatments. Each of the clusters had a fairly even distribution of each treatment in it, even though it seemed to be doing a good job of placing similar phenotypes together. We wondered if using too many features in our clustering was an issue, so we reduce the dimensions of each point with *principal component analysis*. However, this did not yield better clustering results than the complete data.

Clustering Separated Cells by Phenotype



Heatmap of average values in each cluster. The y-axis contains the clusters, and the x-axis contains the different features.

Clustering Failed to Separate Treatments

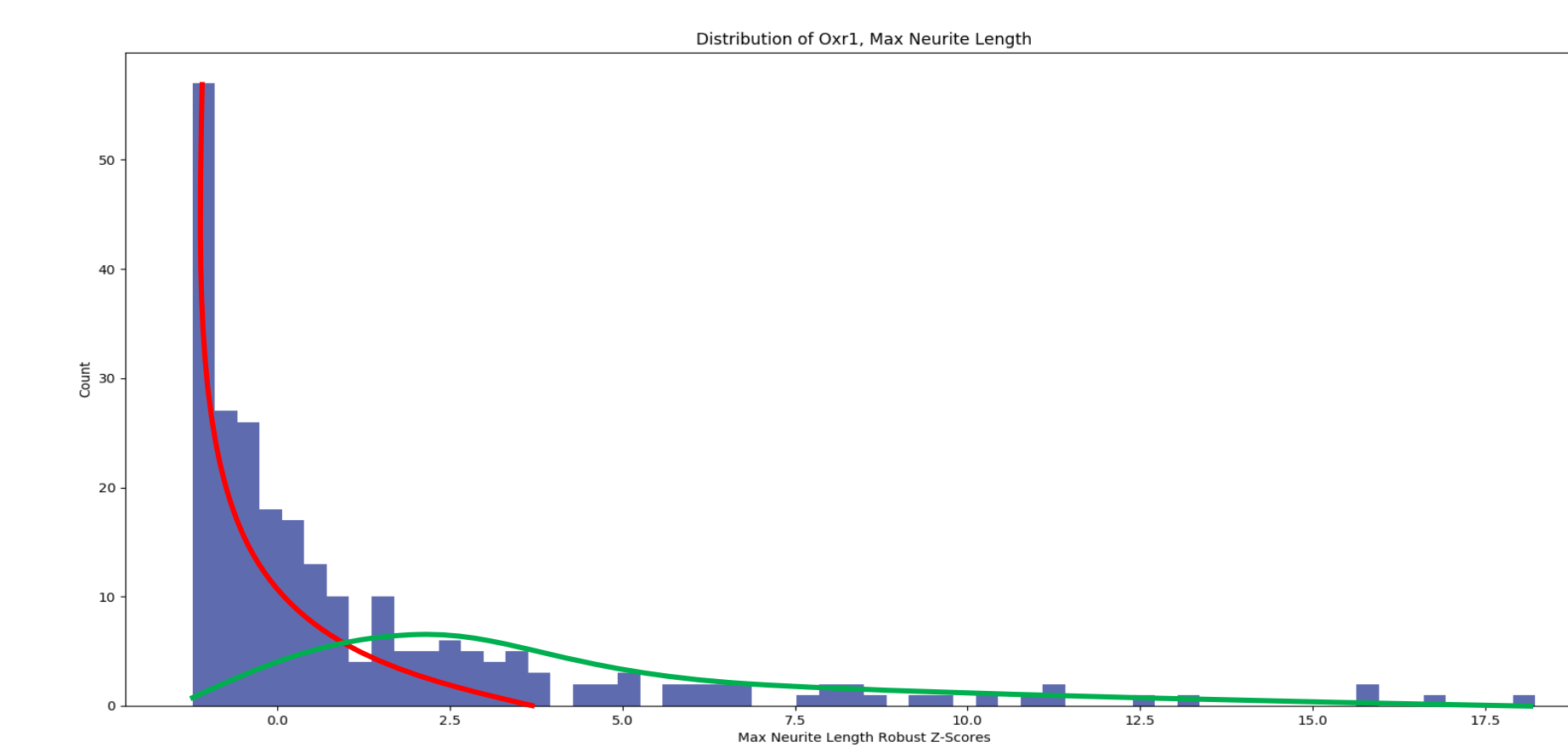


Heatmap of percent of treatment in each cluster. The y-axis contains the clusters, and the x-axis contains the treatments.

Distribution Observation and Thresholding

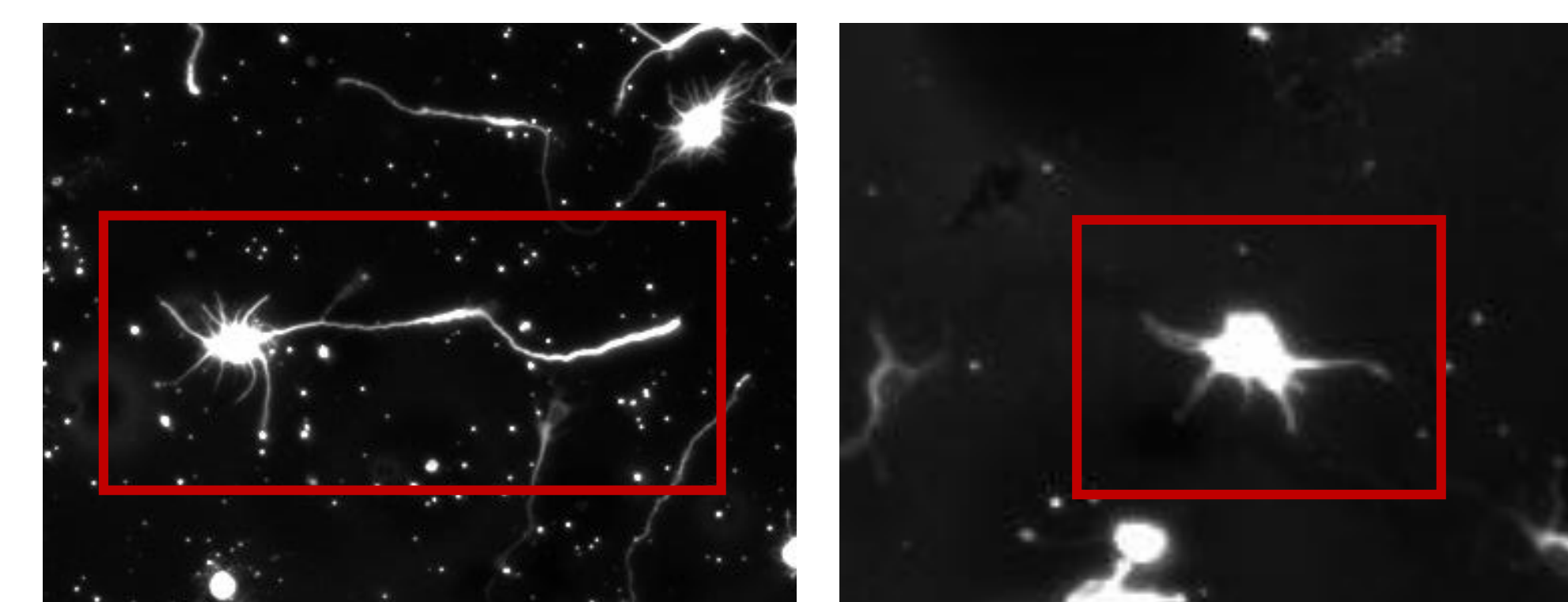
We hypothesized that the cells could be classified as either *responders* or *nonresponders*. Regardless of treatment group, some cells seemed to respond very well to the treatment and would grow very long neurites, and others seemed to hardly respond at all. We observed a relatively consistent cutoff value in the *maximum neurite length*, and what seemed to be a subtle bimodal distribution. The groups appeared to be overlapping quite heavily, so we removed the values that were close to the threshold, and split each group into *responder* cells and *nonresponder* cells. Through clustering, we confirmed these two groups had distinct phenotypes.

Bimodal Distribution in Growth



Subtle bimodal distribution in control group's *neurite maximum length*. The red drawn-on curve represents our idea of the non-responders group, and the green represents the responders.

Responder versus Nonresponder

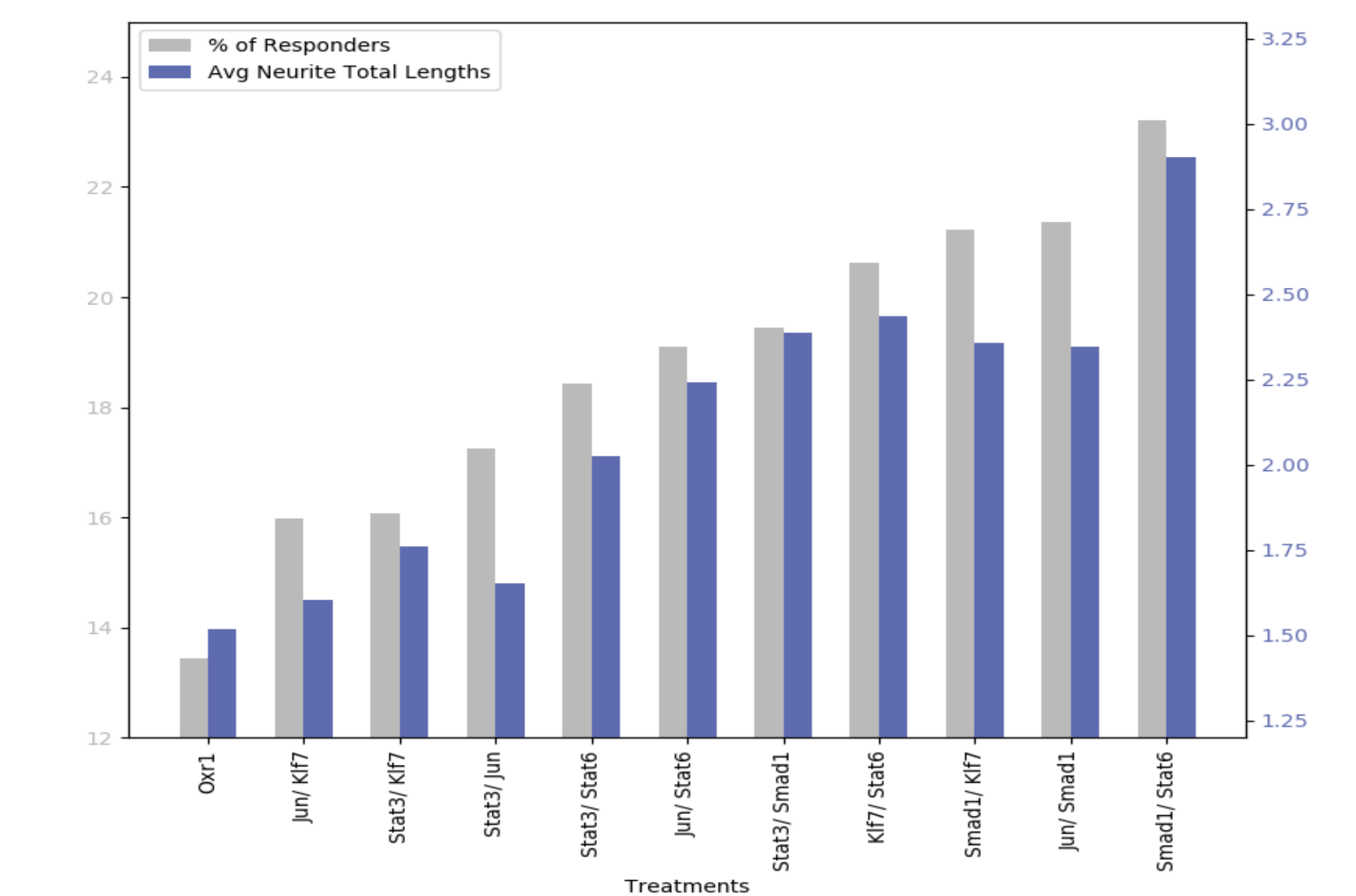


Average responder (left) in comparison to average nonresponder (right). Both images from Stat3/Smad1 treatment, but responders and nonresponder phenotypes consistent among treatment groups.

Percentage of Responders is Significant

We were unable to observe phenotypic differences in the responders of each group by clustering, or by analyzing the average values of each group. Instead, there was a very clear correlation between the percentage of responders in a given group, and that group's average values for neurite growth.

Correlation: % of Responders and Average Growth



Correlation between the percentage of responders in treatment group, and metric for average neurite growth measured in the past.

Conclusion

The response of rat cortical neurons to different treatments was found to be more complicated than a simple shift in the average neurite growth. The results show that different treatments will vary the percentage of cells that respond to the treatment once transfected. We can also see that there is a correlation between the percentage of responders in each group, and the average values for neurite growth for all transfect cells in a group. It is more than likely that the average values are a result of the percentage of responders in a given group.

Summary

The goal of this project was to perform a more in-depth analysis of data involving transcription factors' effects on neurite growth in vitro. After failing to observe distinct phenotypes of cells in each treatment group, we were able to differentiate *responders* and *nonresponders* in each treatment group, and noted a correlation between the percentage of *responders* in each group and the values for average neurite growth per group gathered in previous analysis.

Acknowledgements

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