The dynamics and regulators of cell fate decisions are revealed by pseudotemporal ordering of single cells

Trapnell, Cole, et al (Nature biotechnology,2014)

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INTRODUCTION

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- Area with ample scope of insights: biological and computational(RNA-Seq is *challenging!*)
- NOTE(A common misbelief): RNA-Seq doesn't measure what is *technically* gene expression: Measures *relative transcript abundances*

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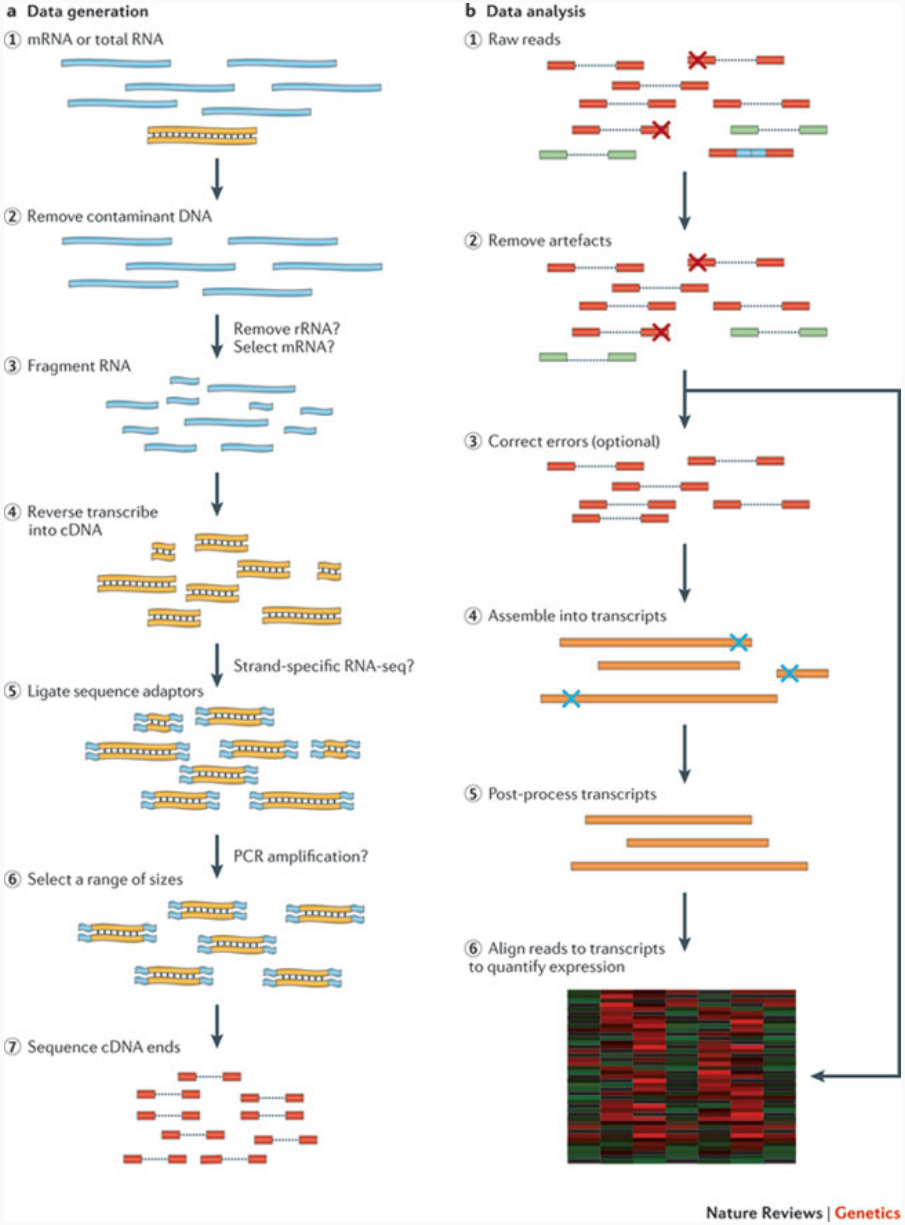

Figure 1: A typical RNA-Seq experiment

[1] Next-generation transcriptome assembly, *Martin et al. Nature Reviews Genetics(2011)*

SINGLE CELL RNA-SEQ MOTIVATION

’ Context: Studying differentiation

Transcriptional dynamics of a temporal process like cell differentiation is challenging

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High variability arises due to high cell-to-cell variability: Simple averages don’t work!

Which is a better treatement?

Treatments for kidney stones

	Treatment A	Treatment B
Small Stones	Group1: **93%(81/87)**	Group2: 87% (234/270)
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DIGRESSION

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Hint: Is your sample size *enough* to draw causality relations?

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Both	78% (273/350)	**83% (289/350)**

Reversal of inequality! : Simpson's Paradox. Sizes of the groups being combined are not same. Large stones patients were offered *better* treatment A, small stones patients were offered treatment B.
$\implies$ Stone size is a 'confounding variable'.

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- Using an unsupervised algorithm 'Monocle', we want to study the temporal development of single cell
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- Cultured in high serum medium, then shifted to low serum $\Longrightarrow$ induces differentiation

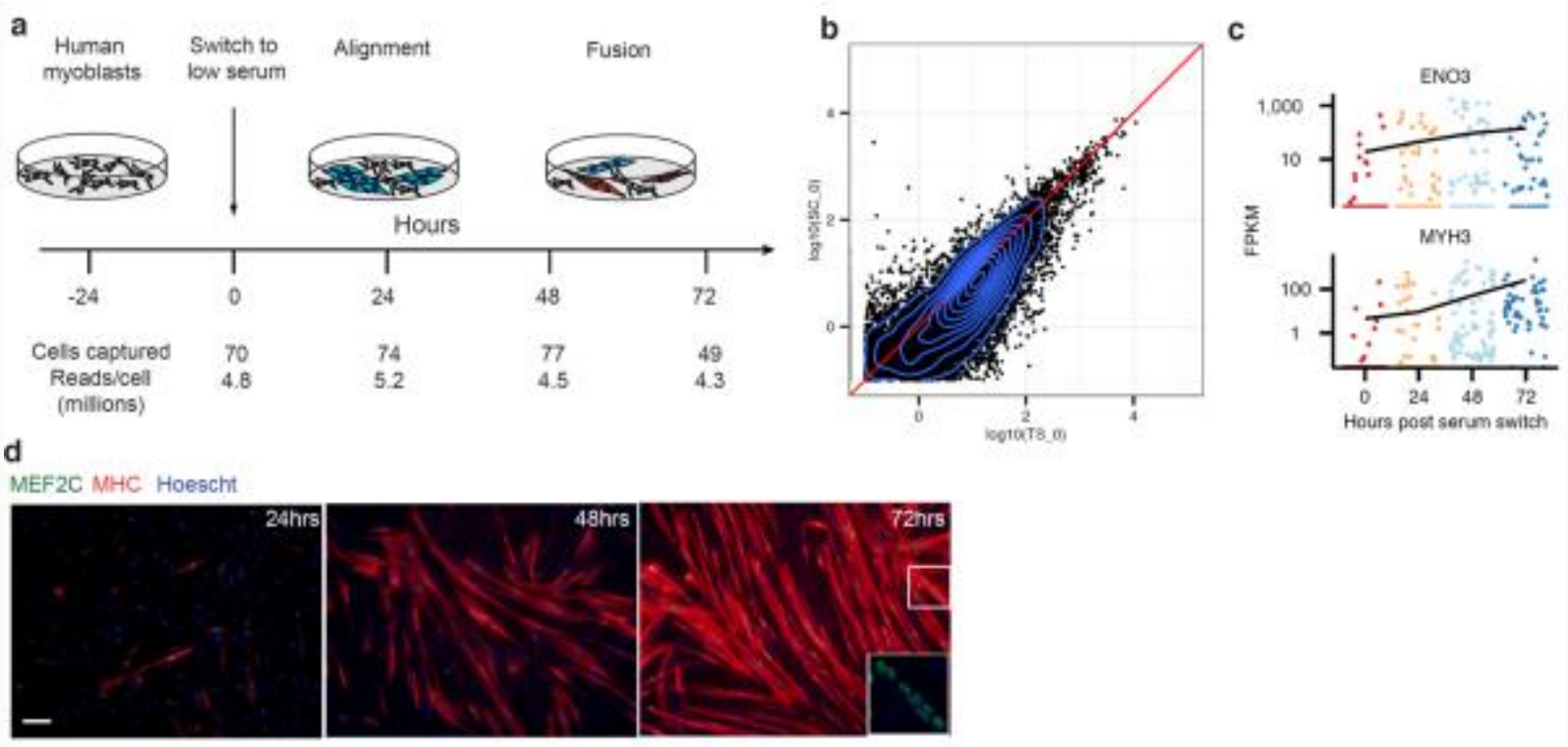

Figure 2: a. Cell Culturing b. Sanity check with bulk RNA-seq(time=0) c.Late stage differentiation markers d.

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- It is possible to create a trajectory using *pseudotime* values: So there are now branches + a main trajectory(ref figure)

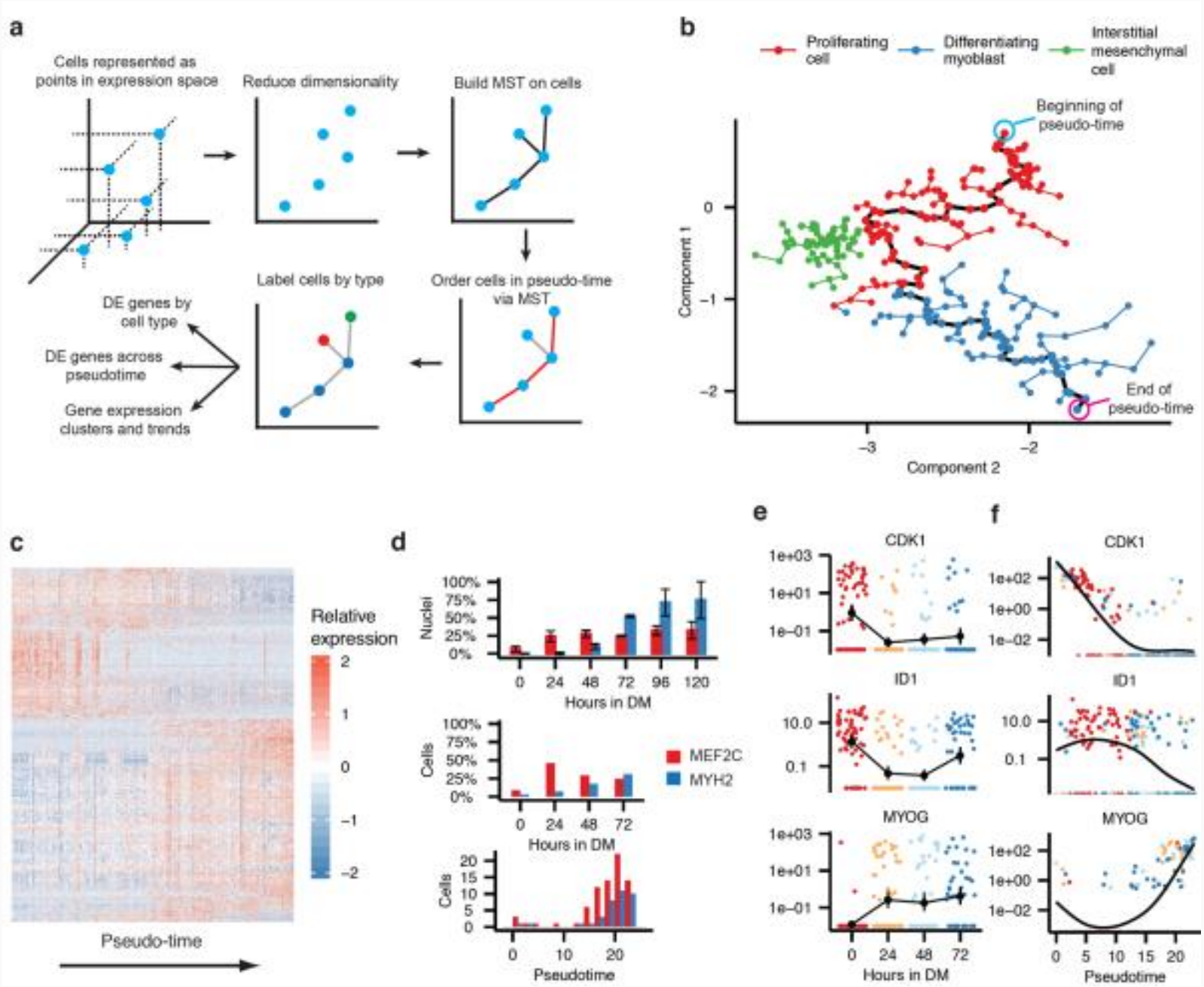

Figure 3: Results

RESULTS

METHOD/RESULTS

- Differentiation = Two phase trajectory(differentiating) + non-differentiating cells
- Trajectory 1: Cells selected under High Mitogen Condition(Mitogen enhances differentiation/division)(time = 0 cells)
- Trajectory 2: 24h, 46h, 72h differentiating cells (MYOG is a known marker for differentiation)
- Trajectory 3: Lacks myogenic markers, possibly did not originate from the myoblasts(known to be stimulants of muscle differentiation)

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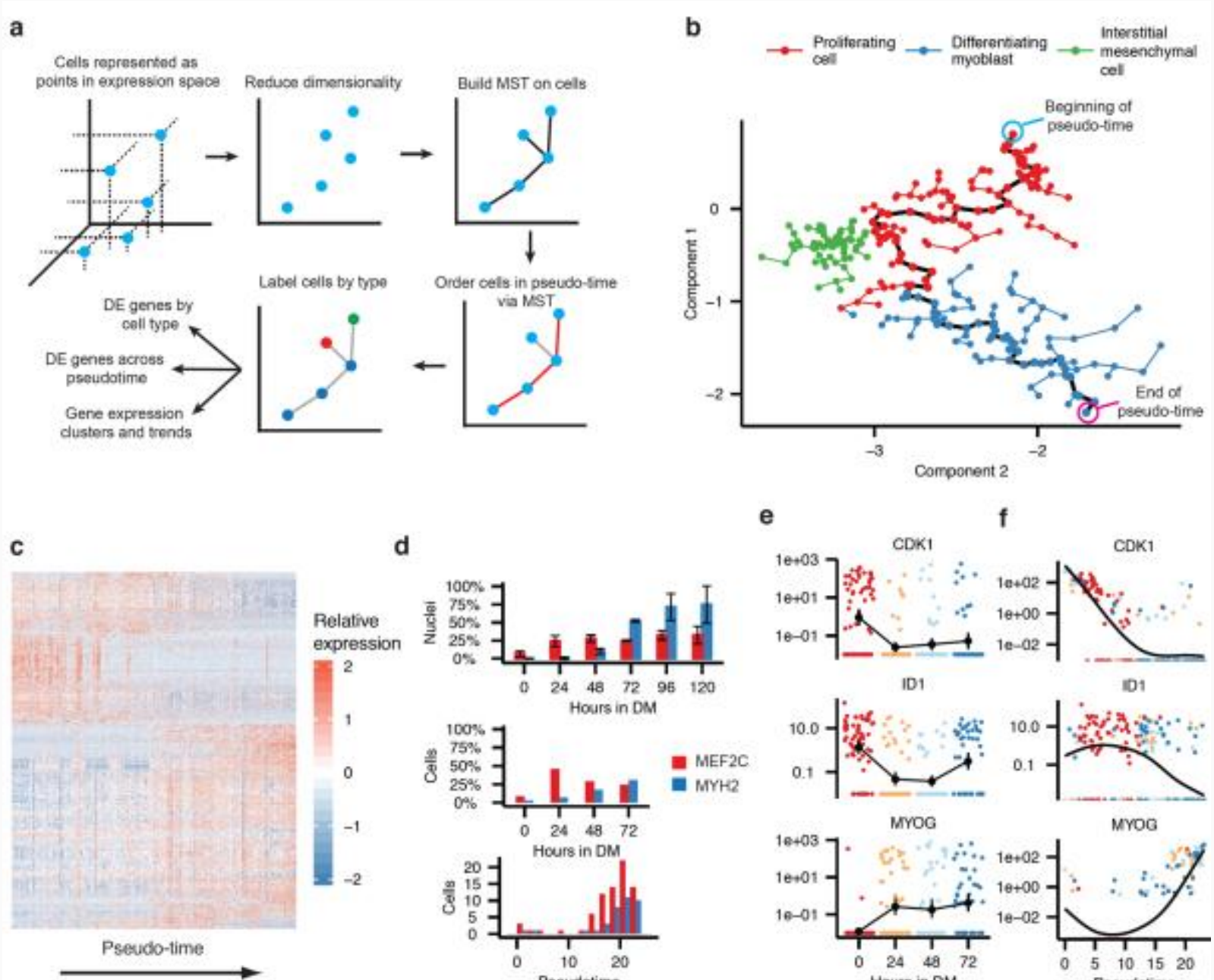

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General Additive models:

$$g(E(y)) = \beta_0 + f_1(x_1) + f_2(x_2) + \cdots + f_m(x_m)$$

where x_i are the predictor variables. and g is link function(log/identity) and Y belong to the exponential family, the advantage over GLM being flexibility with nonparametric fits. (ofcourse, less interpretable than GLM)

MORE VALIDATION

- A further validation step involved *clustering* genes with *[similar]* expression levels, with the assumption **similar trends in expression = similar biological function**
- Genes downregulated early or upregulated late were found to be GO enriched in myosis, cell cycle-exit, activation of muscle specific proteins

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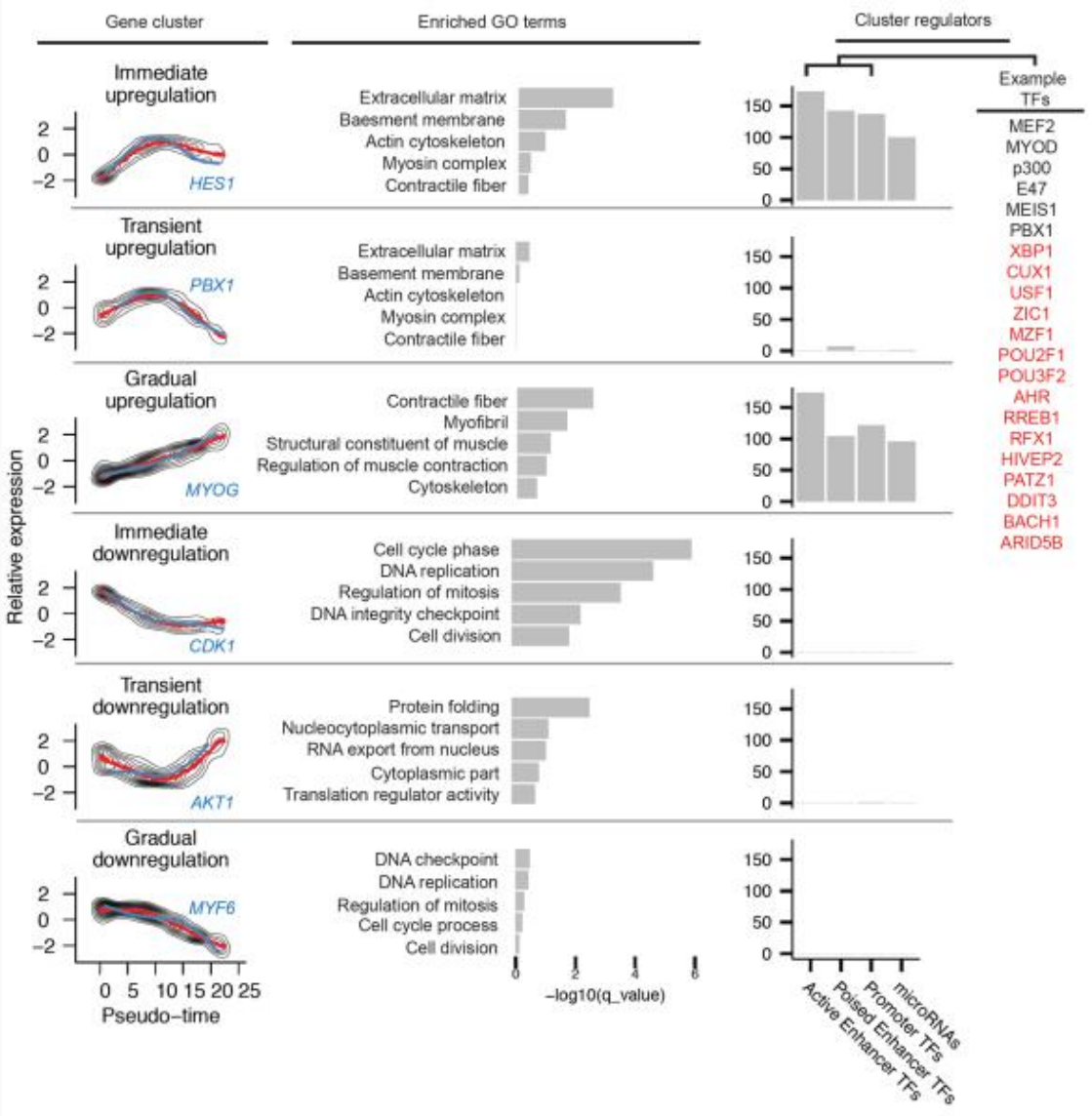

Figure 4: GO analysis of gene clusters

CONCLUSION

SUMMARY

- Monocle takes an unsupervised approach to determine the temporal trajectory for differentiating cells
- Makes it possible to identify the *latent variables* that often get shadwoed with bulk RNA-Seq studies
- The GO validation step is not very convincing, the method otherwise looks solid(there were more experimental validations performed)

Yanai, Itai, et al. "Similar gene expression profiles do not imply similar tissue functions." TRENDS in Genetics 22.3 (2006): 132-138.

Questions?