

# Transcriptomic Analysis of Glioma Cells

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## Introduction

### Glioblastoma Multiforme (GBM)

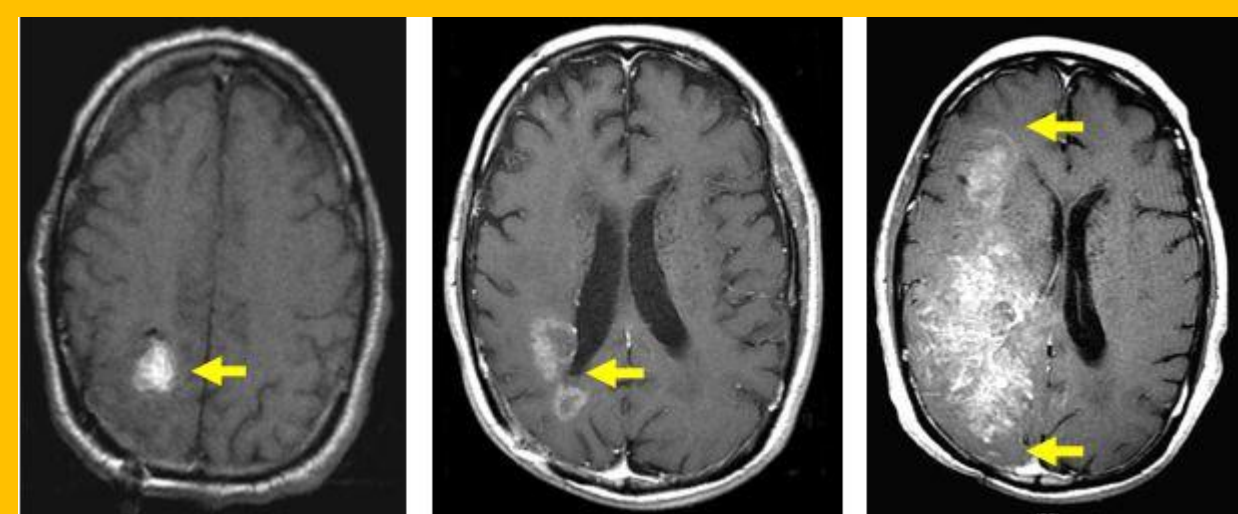


Fig. 1

- Form of Brain cancer with worst prognostic outcome

### Adhesion genes and pathways

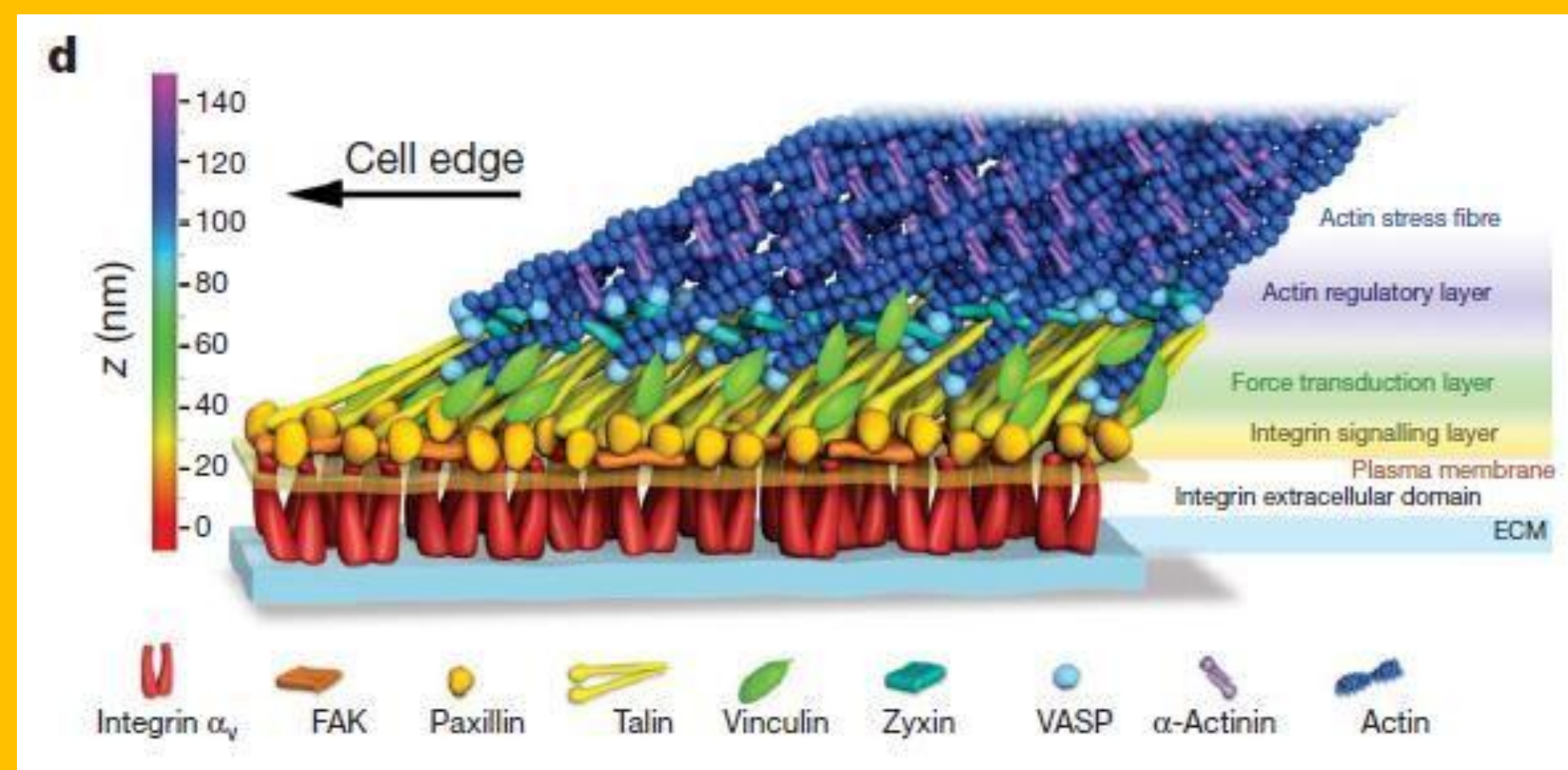


Fig. 2 (1)

- Layer of genes involved in cell motility
- Of key interest when studying movement of GBM U251 cells on substrate

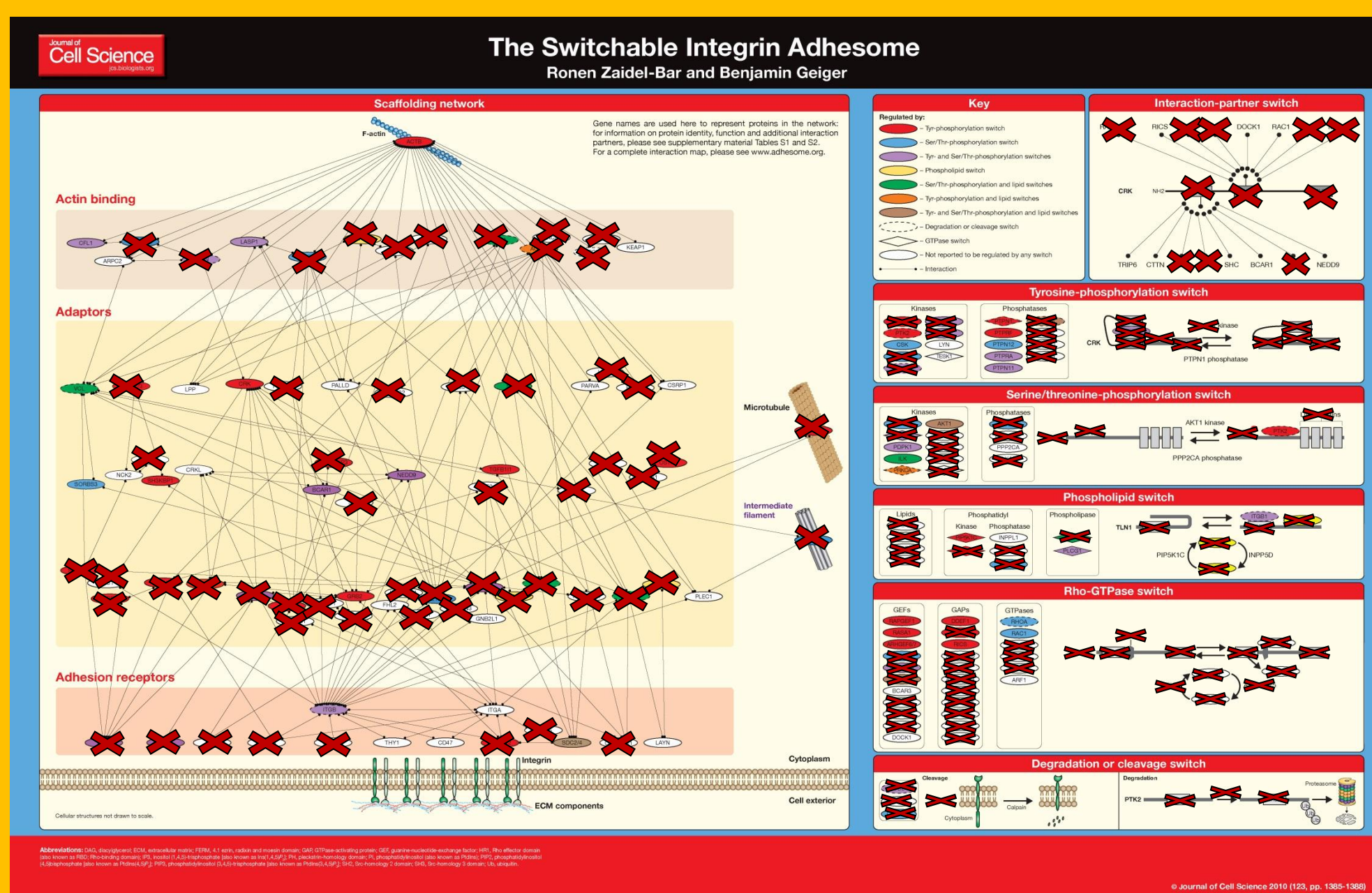


Fig. 3 (2)

- GBM moves according to the motor clutch model
- Many genes of particular interest in motor clutch model are also correlated with the adhesome template (fig. 3)

## Methods



Fig. 4 (3)

### Silanizing, Casting, Coating

- 9 Gels: 12, 260, 2000 kPa
- 3 Plastic controls (T25 flask)



Fig. 5 (4)

### Culturing, RNA Purification

- Grow in flask (70-100%)
- Culture cells for 1-2 more days
  - 3 plastic flask (1 per sample)
  - Gels (3 dishes per sample)
- RNA purification using Qiagen kit

## Results

### Genes by fold change analysis

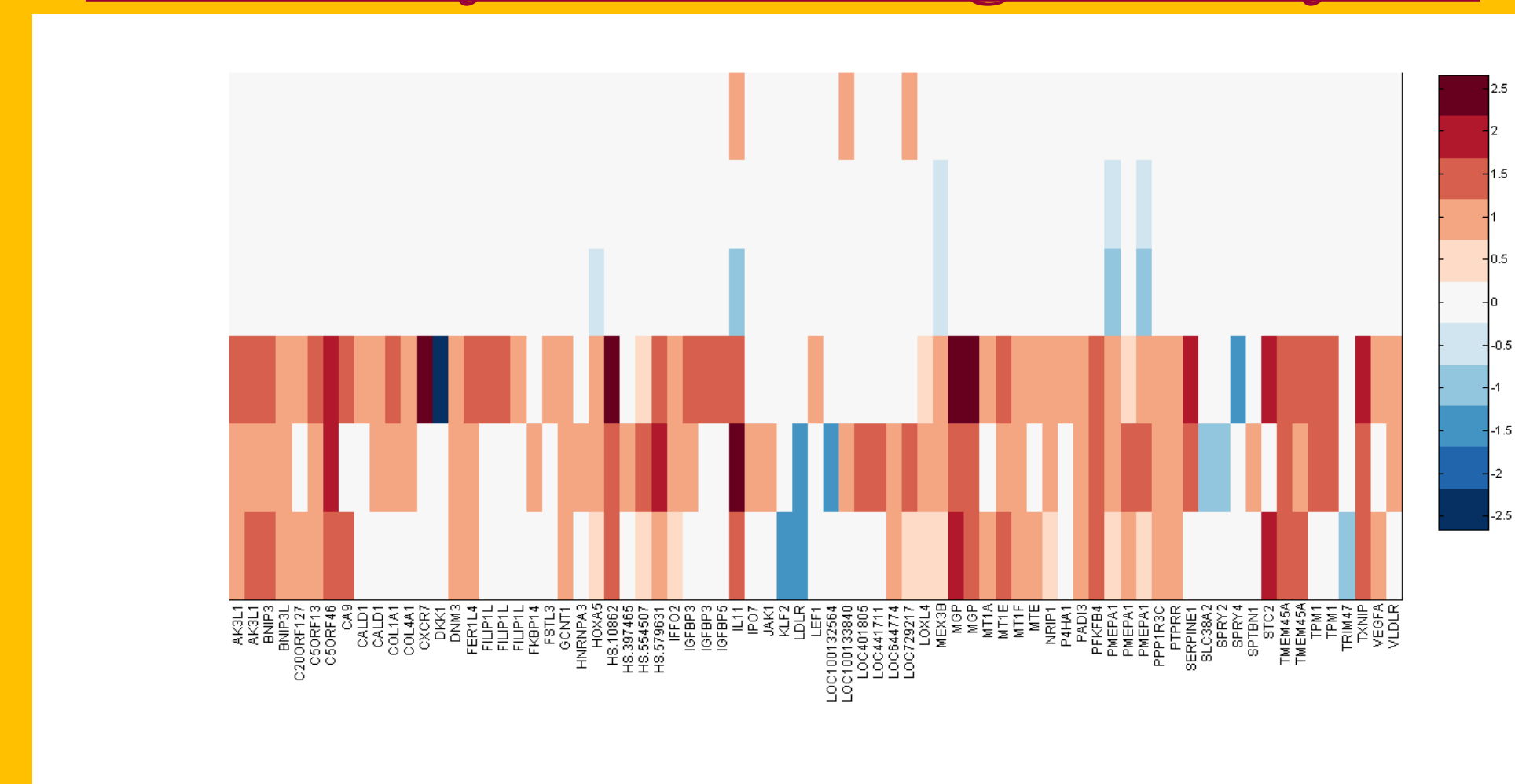


Fig. 6

### Log histogram of key motor clutch genes

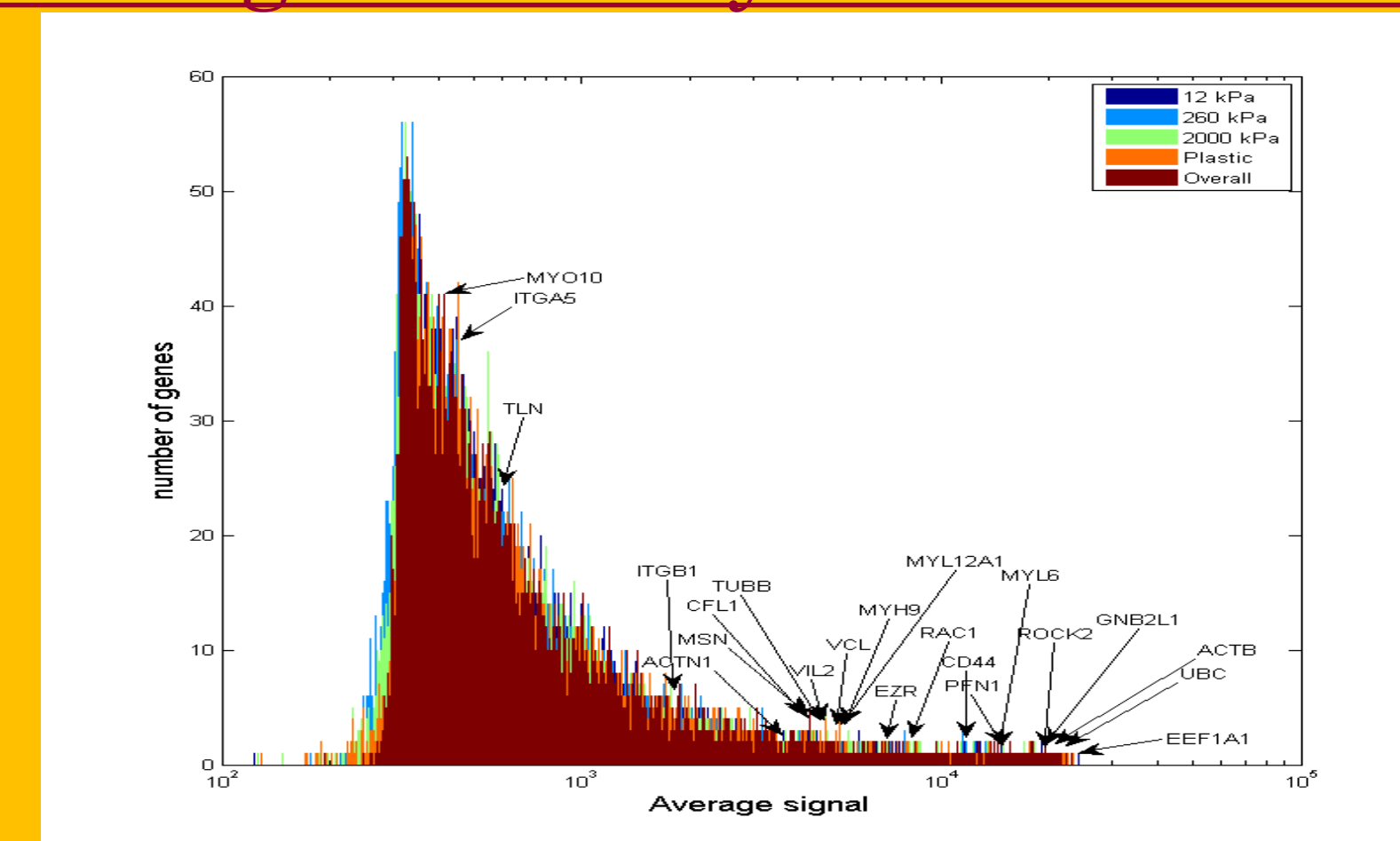


Fig. 7

- Analysis via matlab
- Fig. 6: genes narrowed by:
  - log-2 fold change
  - mult-compare p-value significance
  - expression level cut-off
- To get: fit data set to histogram and only analyze genes above 2 standard deviations from the mean

## Results

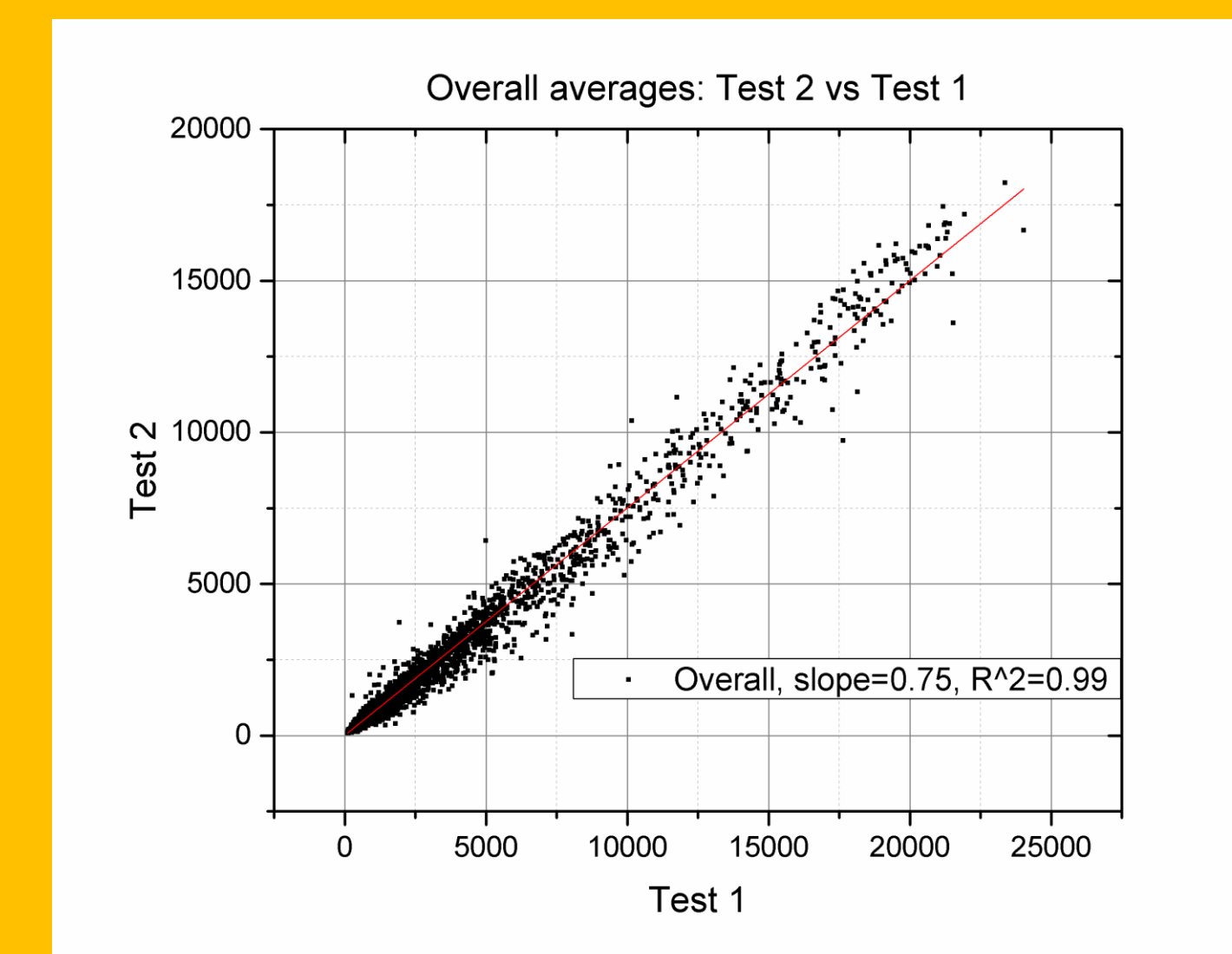
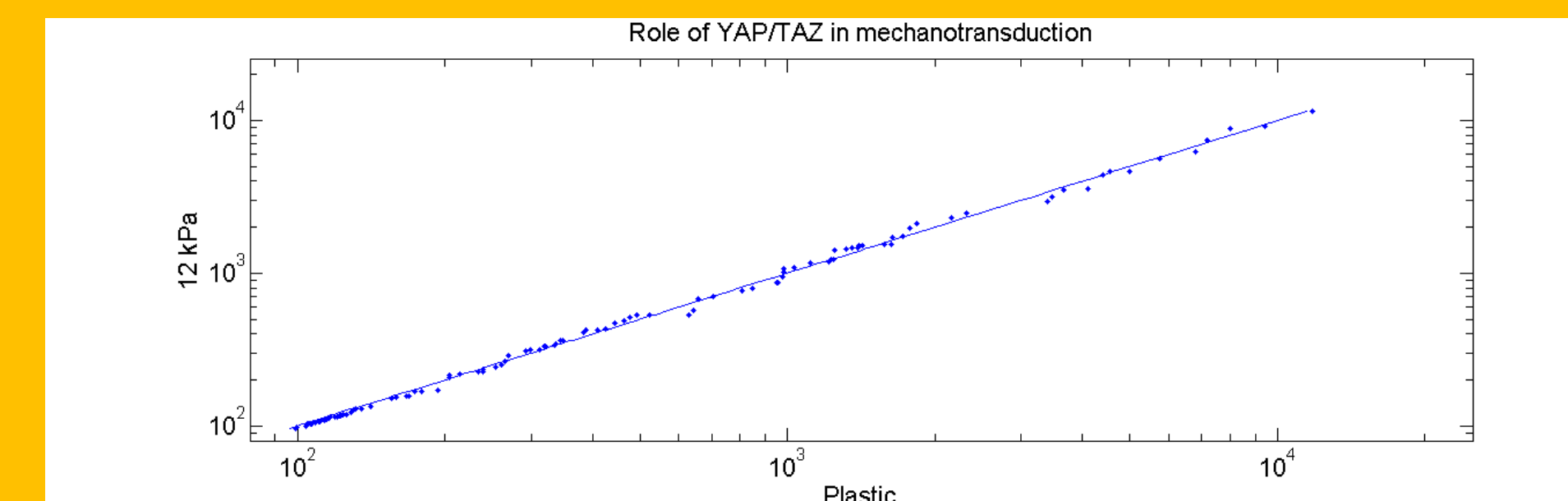


Fig. 8



YAP/TAZ Pathway

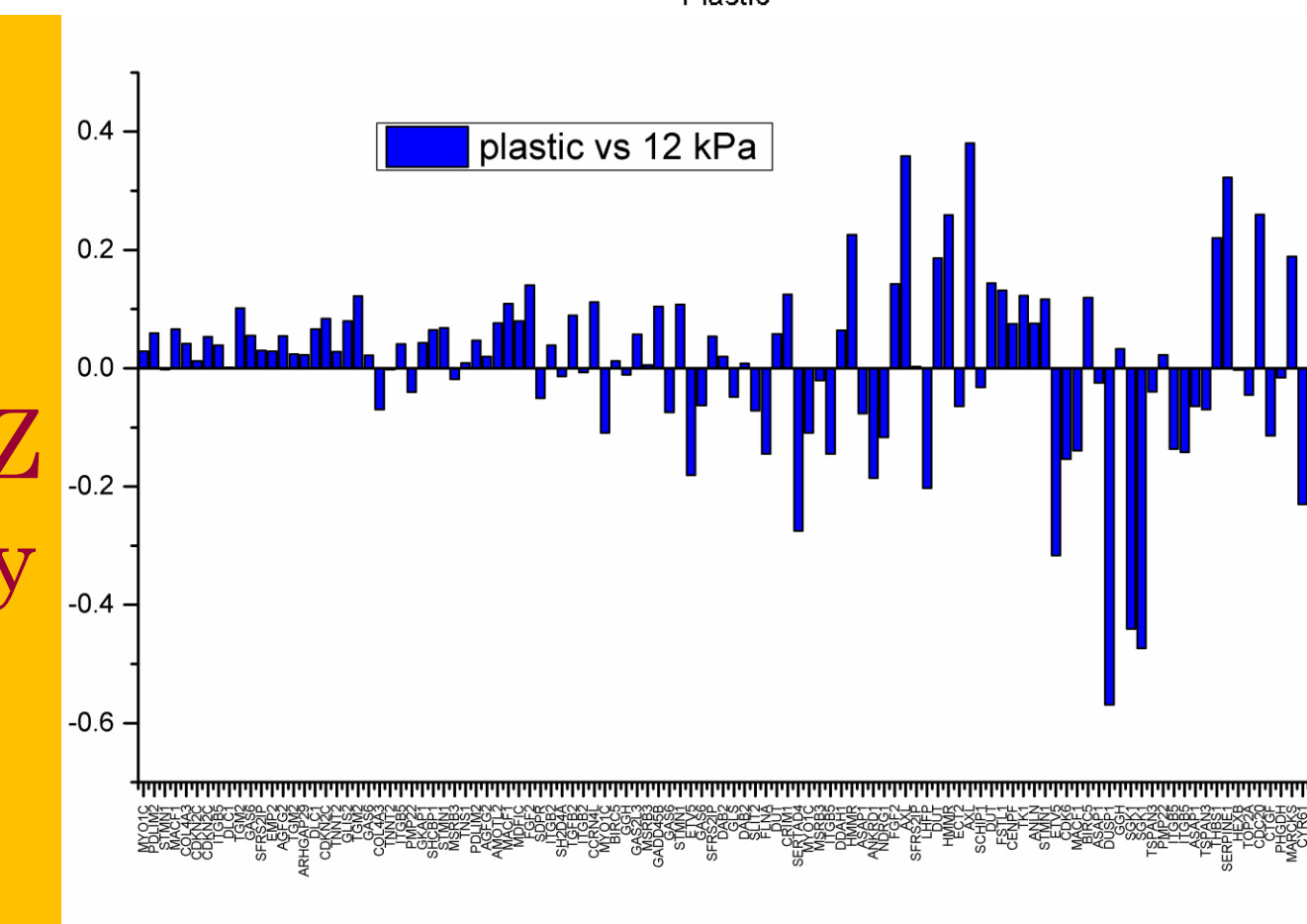


Fig. 9

Random Set of Genes

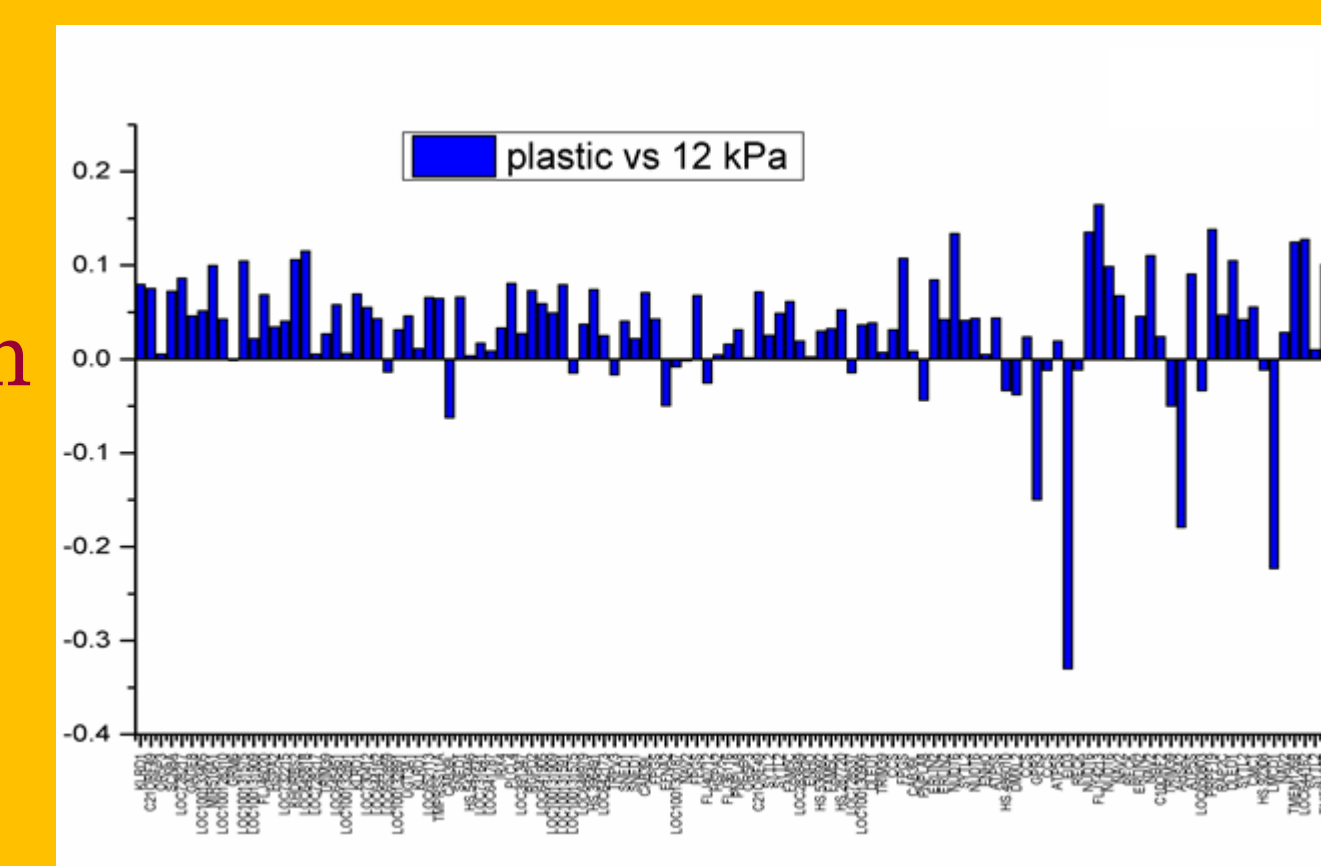


Fig. 10

- Fig. 8 shows that test 1 and test 2 were very well correlated for every gene, but test 2 on average had lower expression values (values are unitless)
- Figures 9 and 10 show that even YAP/TAZ, an important mechanotransduction pathway, does not look significantly different from random set

## Conclusions

- Expression of genes is unaffected by stiffness of gel range chosen
- Genes assumed to have a role in progression of GBM were found to be well-expressed
- The experiment is repeatable as shown by fig. 8

### Issues from tests to address

- Differential expression of genes in the low-expression regime are part of “noise”, need a better description of these genes
- Required high cell densities (and therefore more cell-cell contact) may have had an effect on expression

## Next Steps

### Proteomic Analysis

- Have transcriptomic data, but genes don't translate 1:1 to corresponding proteins
- Mass spectrometry is the option that is most comparable to microarray in terms of output

## Resources

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2. Zidel-Bar, R; Geiger, B. The switchable integrin adhesome. Cell Science 2010; 1385-1388.
3. “Nunc Lab-Tek II Chamber Slide System.” Nunc Lab-Tek II Chamber Slide System. N.p., n.d. Web. 7 Apr. 2014. <<http://www.thermoscientific.com/en/product/nunc-lab-tek-ii-chamber-slide-system.html>>
4. Rneasy Mini Kit. Qiagen. N.p., n.d. Web. 7 Apr. 2014. <<http://www.qiagen.com/products/catalog/sample-technologies/rna-sample-technologies/total-rna/rneasy-mini-kit>>.
5. Dupont, S; et. Al. Role of YAP/TAZ in mechanotransduction. Nature 2011;474:179-183.