

*The time has come:  
SINGLE CELL western  
blotting*

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TJC  
08.07.2014



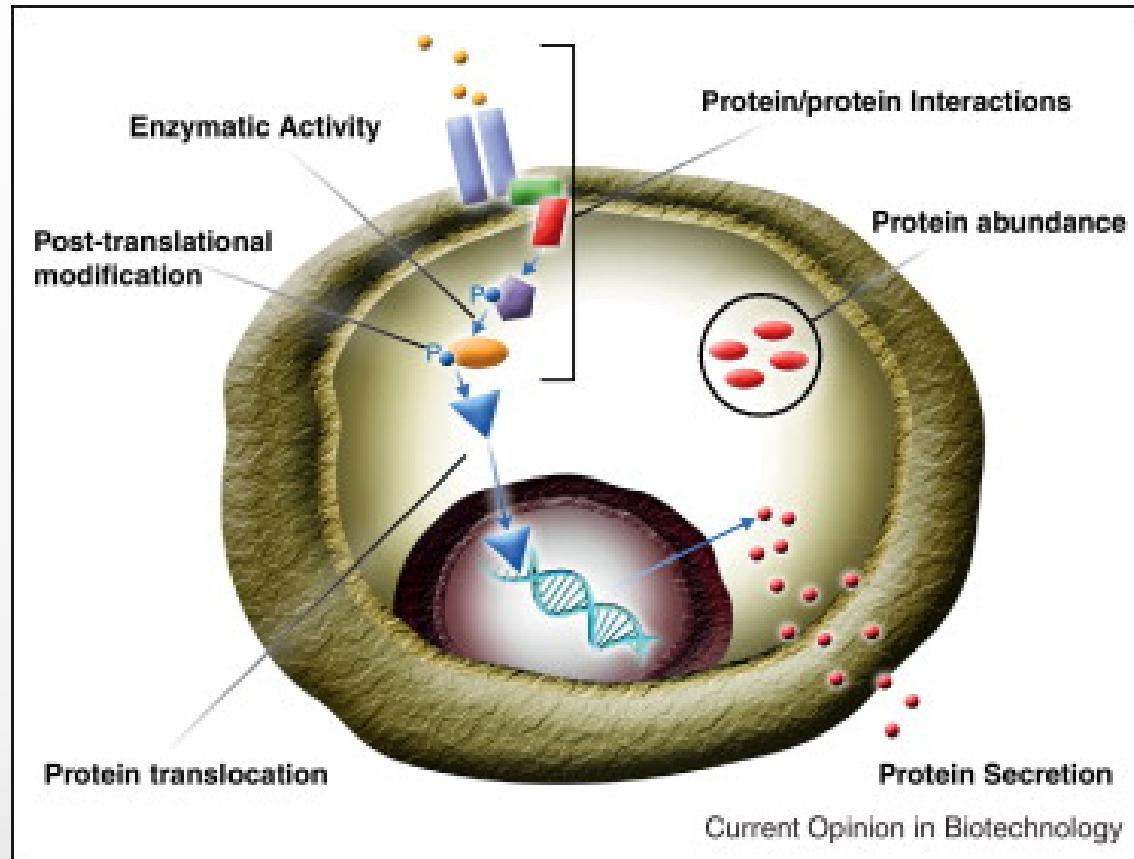
To fully understand diverse and often rare behaviors in complex cell populations, researchers need analytical tools that:

- are optimized for protein analysis of many cells; **Cell heterogeneity**
- offer single-cell resolution;
- provide quantitative and highly specific detection of target proteins;
- do not employ labels that may perturb protein and cell function

GENES

PROTEINS





The biggest challenges to measure proteins in single cells are the small amount of protein in a single cell and the enormous complexity of the proteome

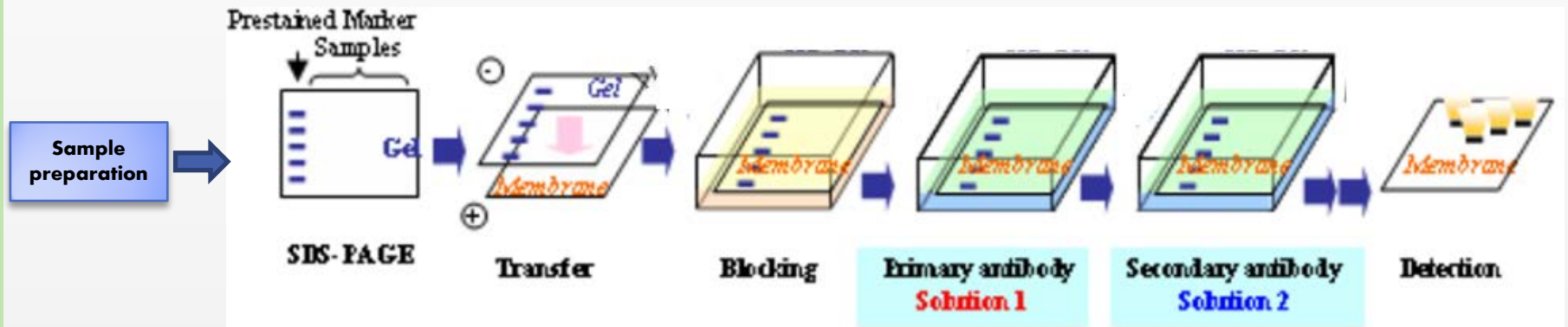
# Single-cell protein analysis

- Flow cytometry (up to 15 proteins simultaneously → profiling of pathways in single cells, analysis of kinases and phosphatases)
- Microfluidic flow cytometry
- Mass cytometry
- Mass spectrometry based single-cell protein analysis

**Single-cell protein analysis techniques lack of resolution, sensitivity or specificity**



# Western blot



AVOIDS THESE PITFALLS BUT IS NOT AMENABLE TO SINGLE-CELL ANALYSIS



NATURE METHODS

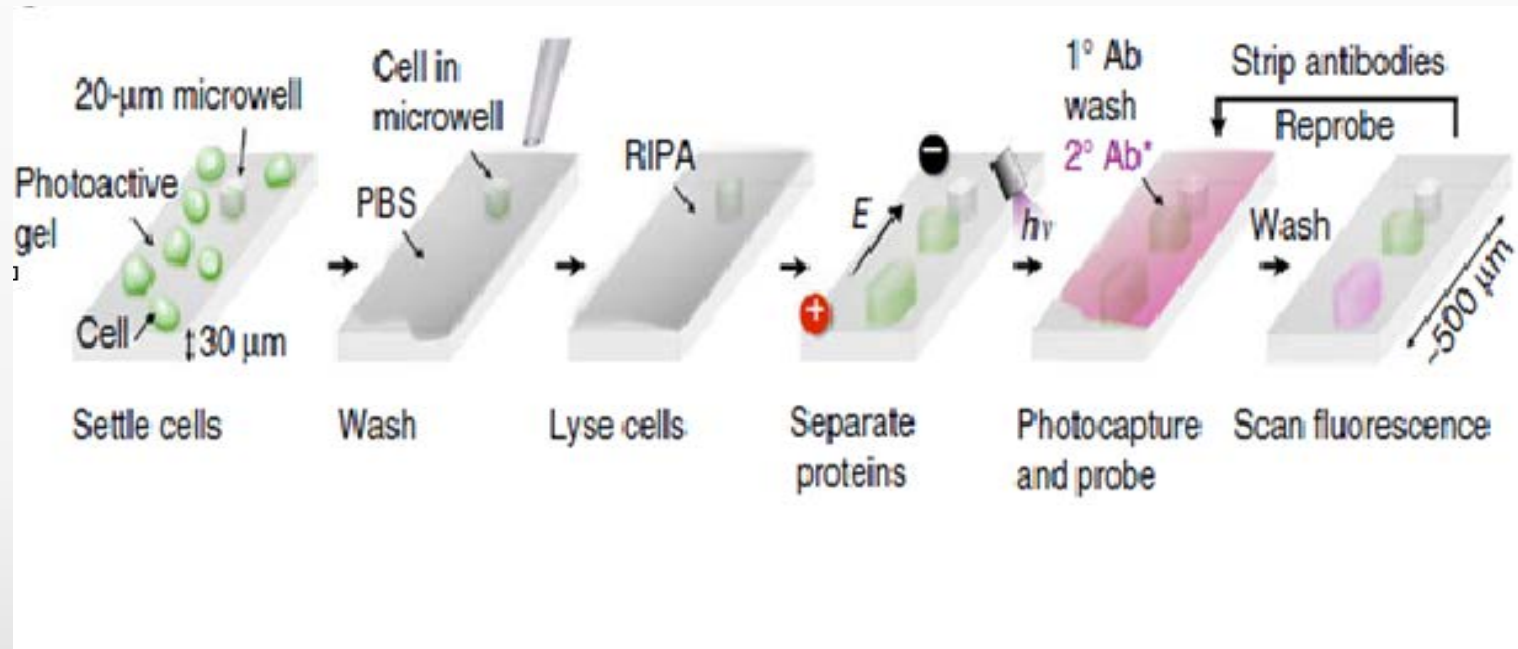
# Single-cell western blotting

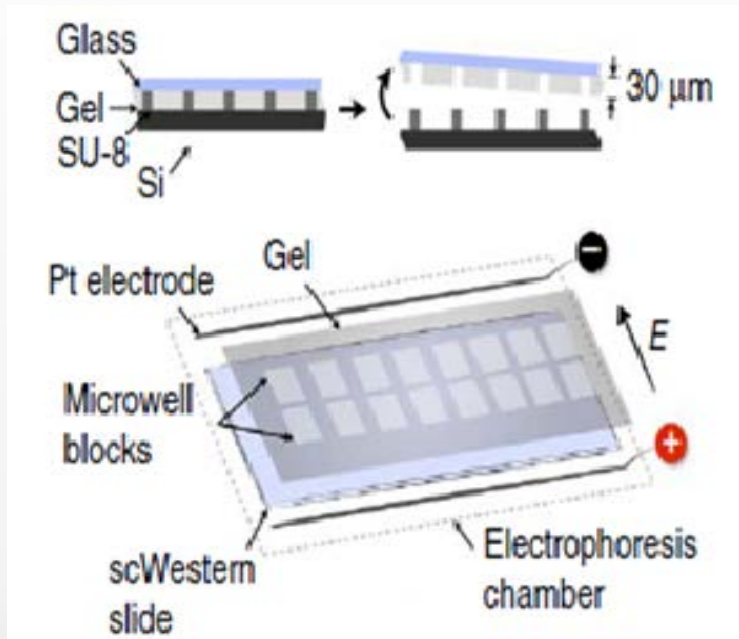
Alex J Hughes<sup>1,2,6,7</sup>, Dawn P Spelke<sup>1-3,7</sup>, Zhuchen Xu<sup>1,2</sup>, Chi-Chih Kang<sup>1,2</sup>, David V Schaffer<sup>1-5</sup> & Amy E Herr<sup>1-3</sup>

1 JUNE 2014



# scWestern





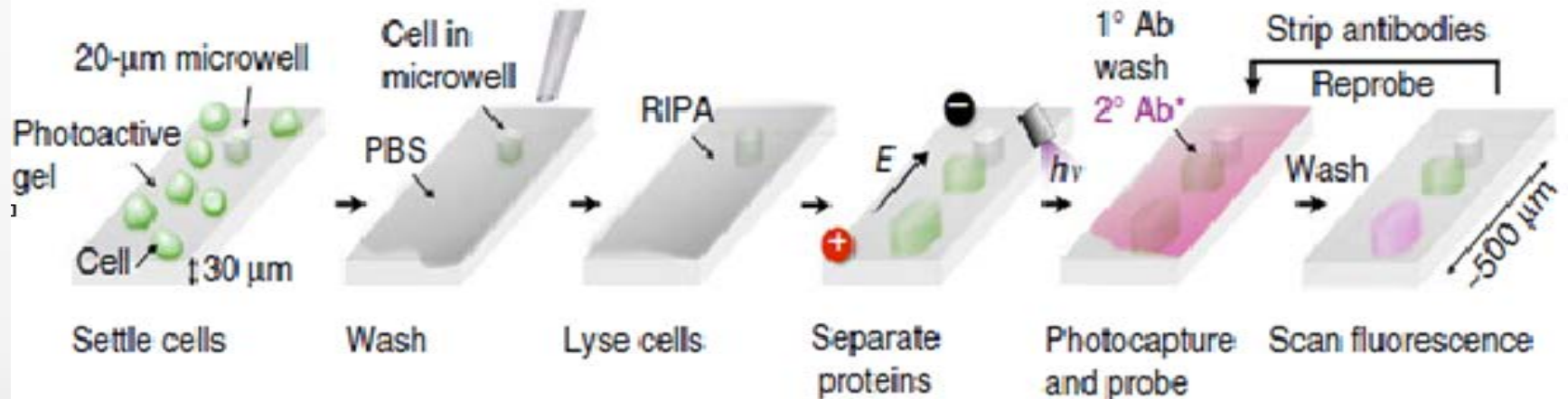
microscope slide  
coated with a 30-μm-  
thin photoactive  
polyacrylamide (PA)  
gel micropatterned  
with an array of  
6,720 microwells



# Design principles of scWestern

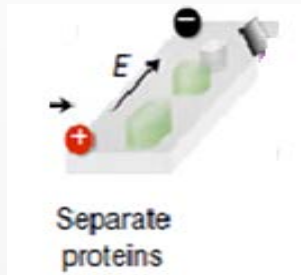
## 1) Fluidic, optical and electrical interfacing

C

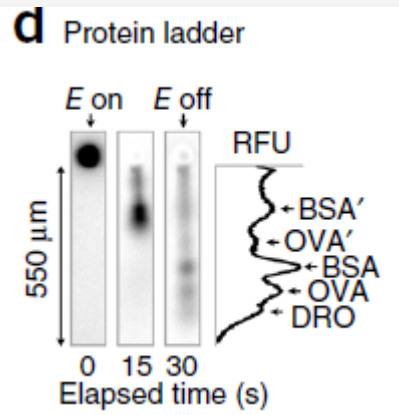


For neural stem cell (NSC) densities of 1,000–1,800 cells per  $\text{mm}^2$  slide area ( $2 \times 10^6$ – $3.5 \times 10^6$  cells in total), they observed single cells in 40–50% of microwells

## 2) High-density scWestern array



after cell lysis, they apply an electric field across the scWestern slide, electrophoresing proteins through the microwell walls and into the thin PA gel sheet



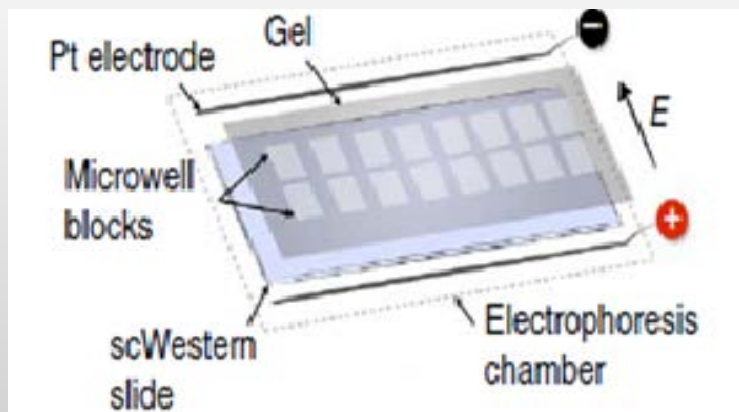
PAGE resolves five fluorescently labeled proteins (27-132kDa) in a 550-μm separation distance

### 3) Protein immobilization and Abs probing



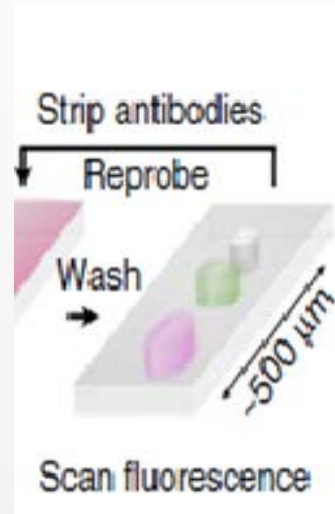
UV-initiated protein immobilization onto the gel

➡ protein immobilization relies on a benzophenone methacrylamide co-monomer cross-linked into the PA gel



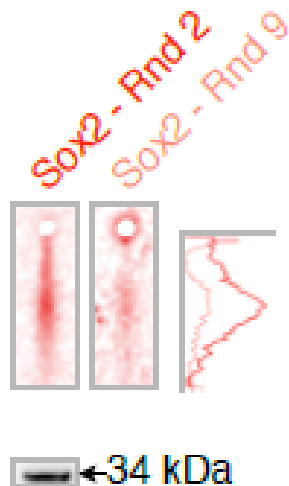
16 assay 'blocks' of 420 microwells each allows application of different antibody solutions to different blocks

## Imaging with a fluorescence microarray scanner

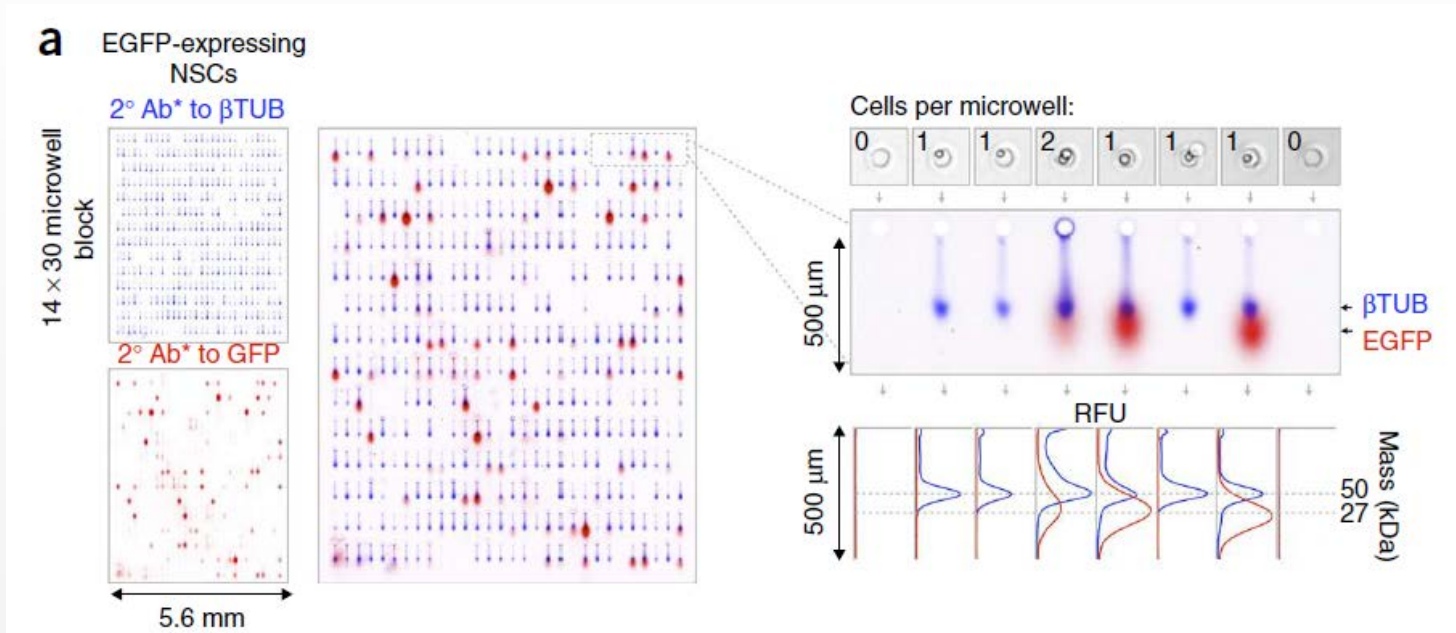


scWestern slides can be stored for long-term archiving and re-analysis of single-cell separations

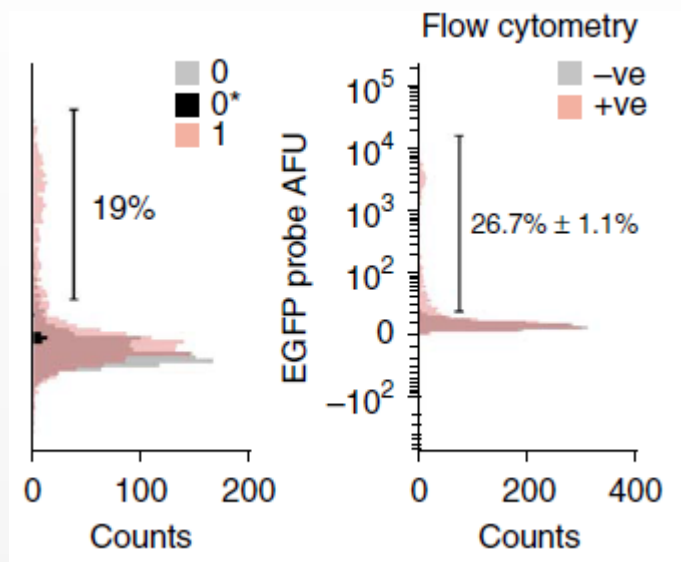
**b**



with a



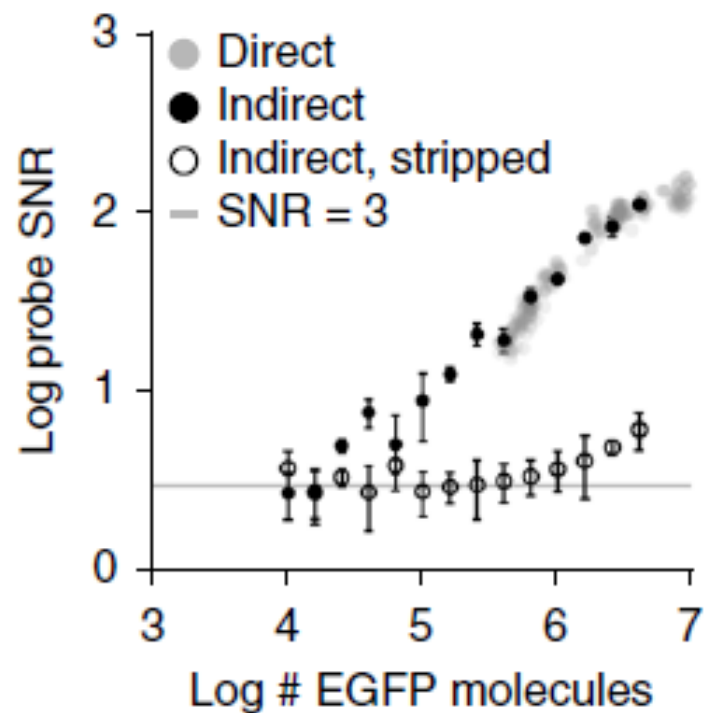
- 12 blocks of a single slide
- 4,128 separations of a possible 5,040 (82%)
- 1,608 separations (39%) came from single cells, on the basis of bright-field microwell occupancy determination (cells per microwell);
- the microwell occupancy running average ranged 0-2.1 cells per microwell with a mean of **1.1 cells** per microwell



**LIMIT OF DETECTION:  
27000 molecules**

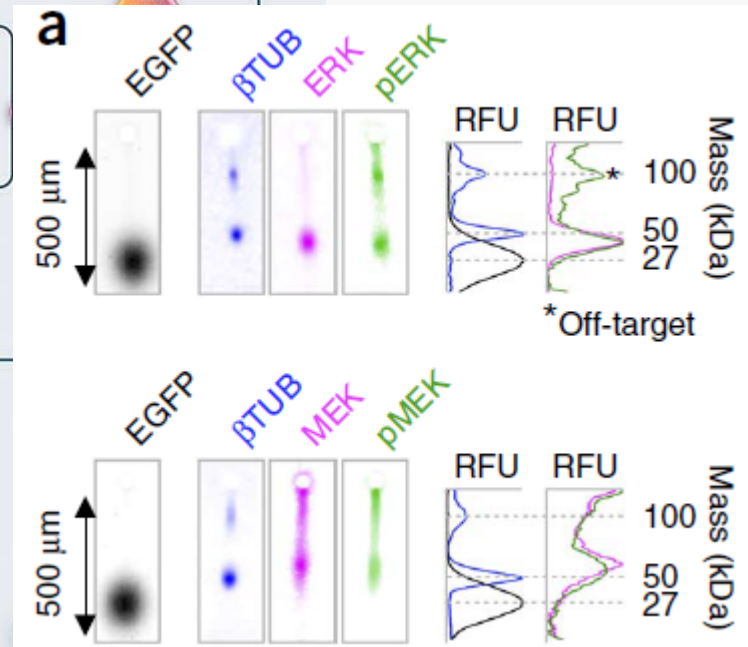
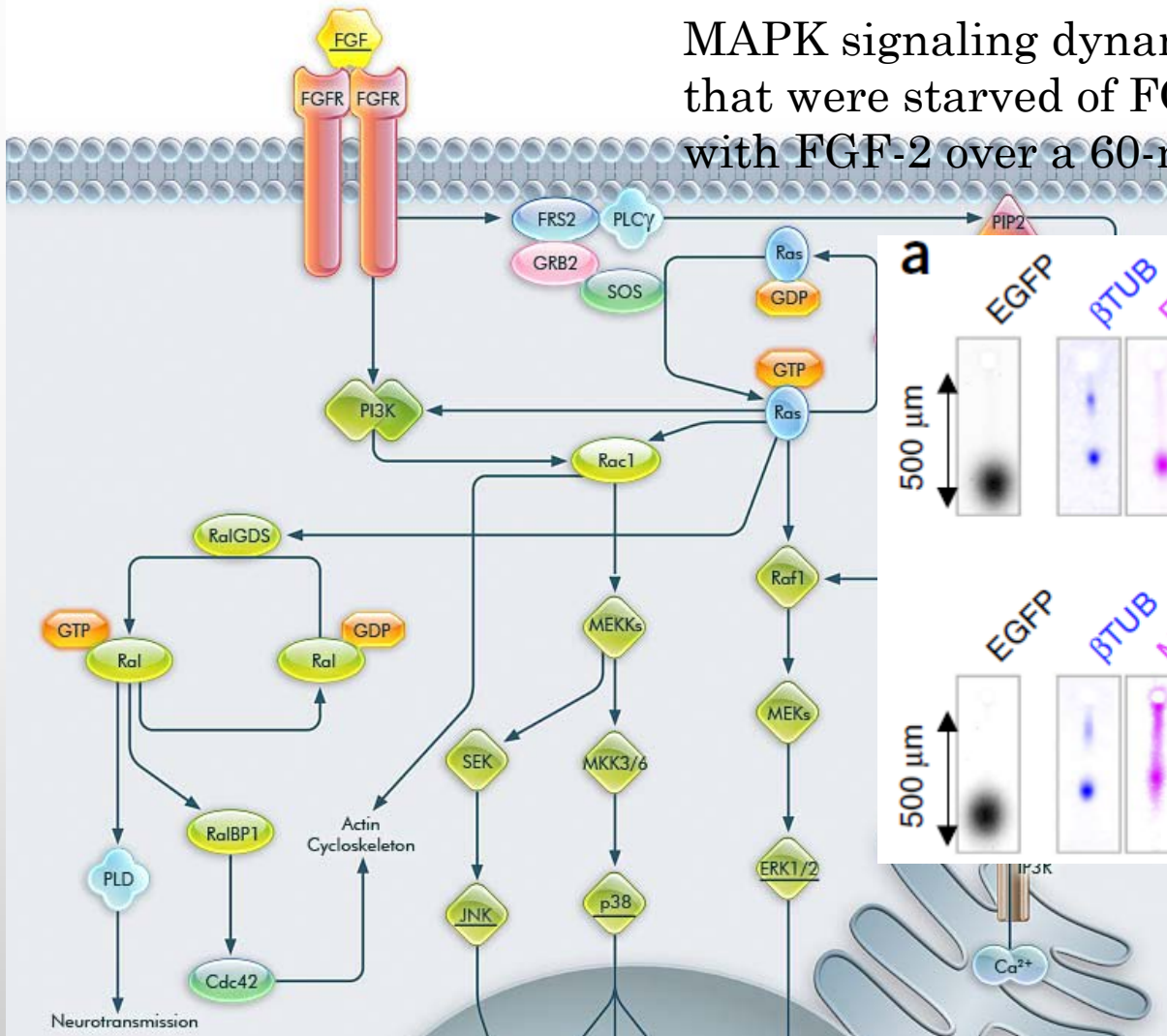
**d**

Calibration curves

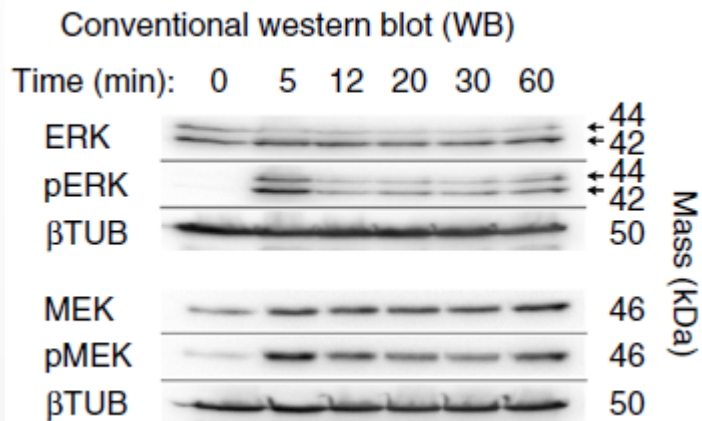


# Heterogeneity in signaling after FGF-2 stimulation of NSCs

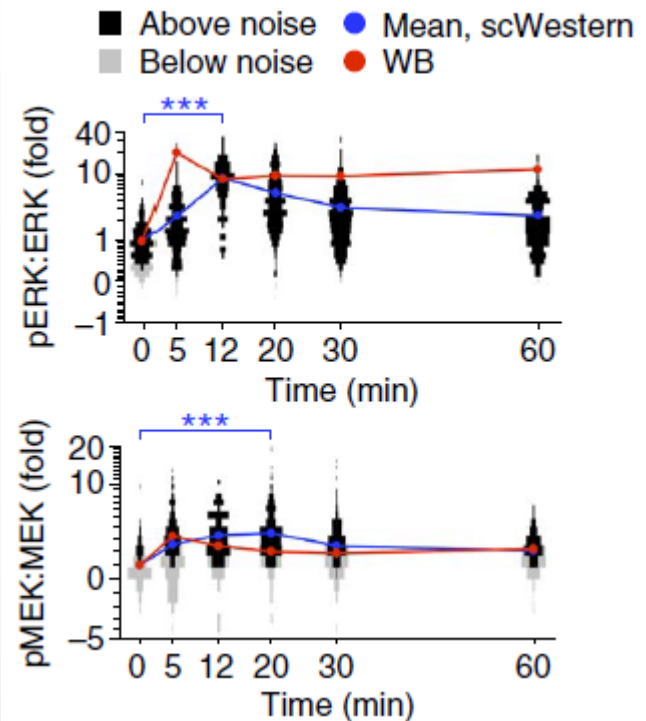
MAPK signaling dynamics within single NSCs that were starved of FGF-2 and stimulated with FGF-2 over a 60-min time course



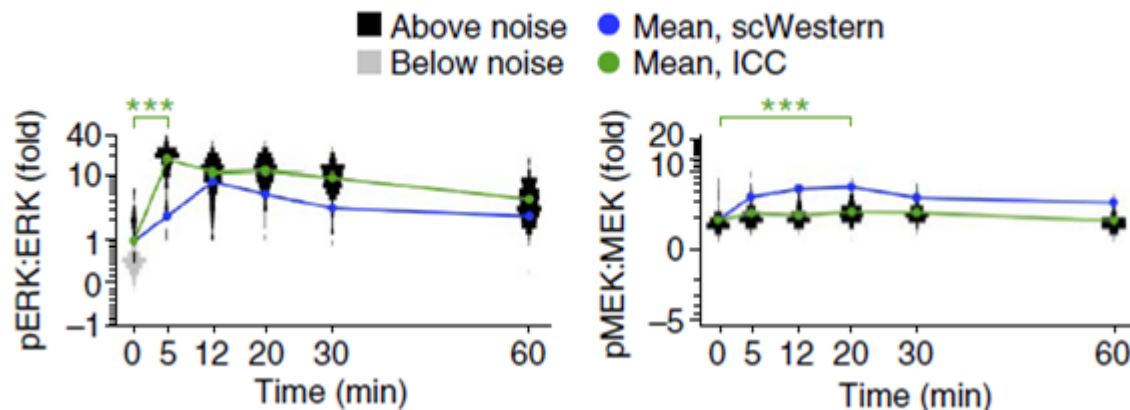




### scWestern

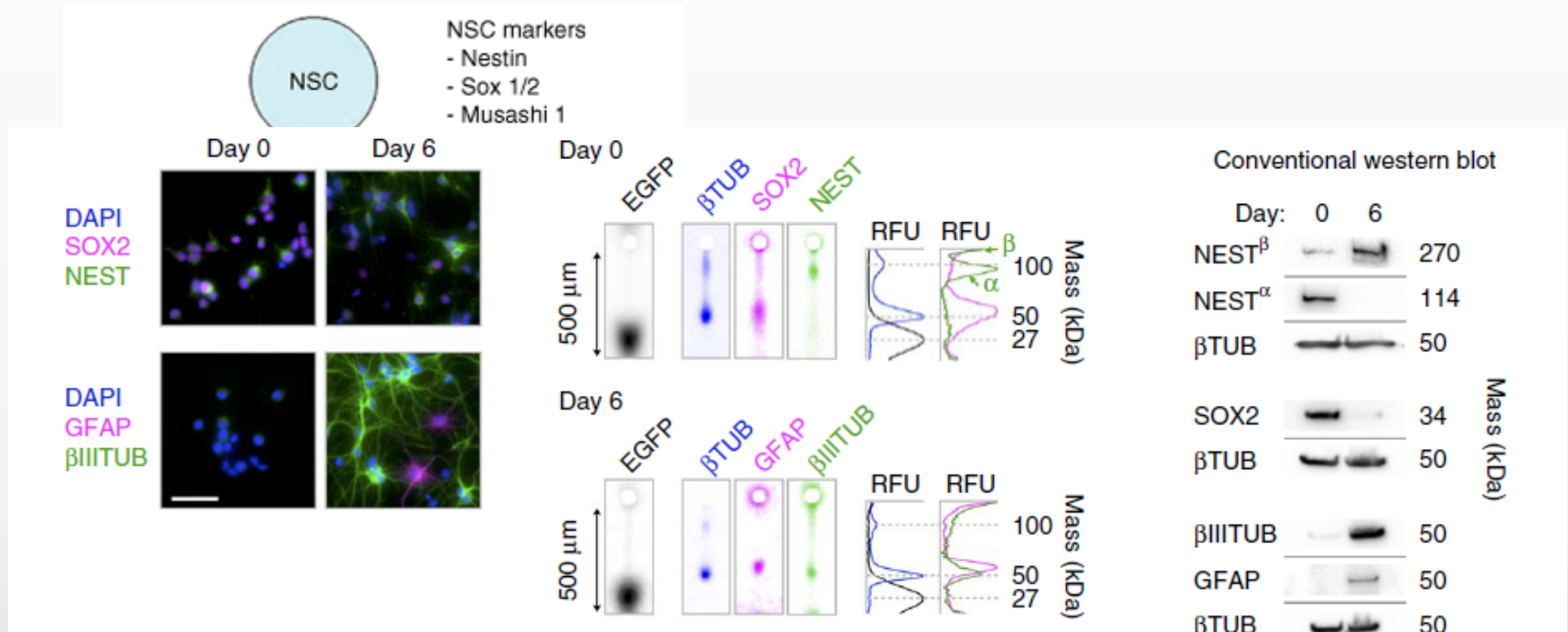


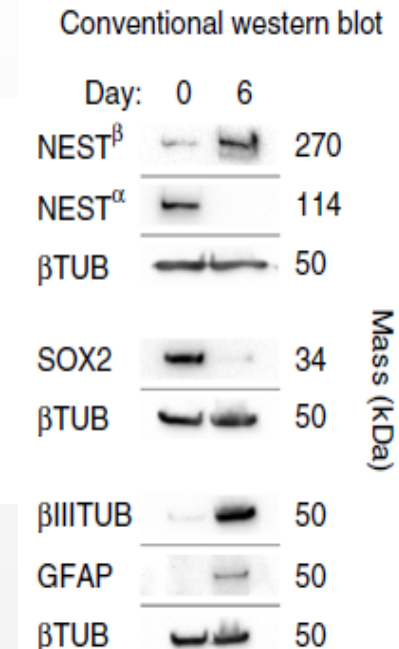
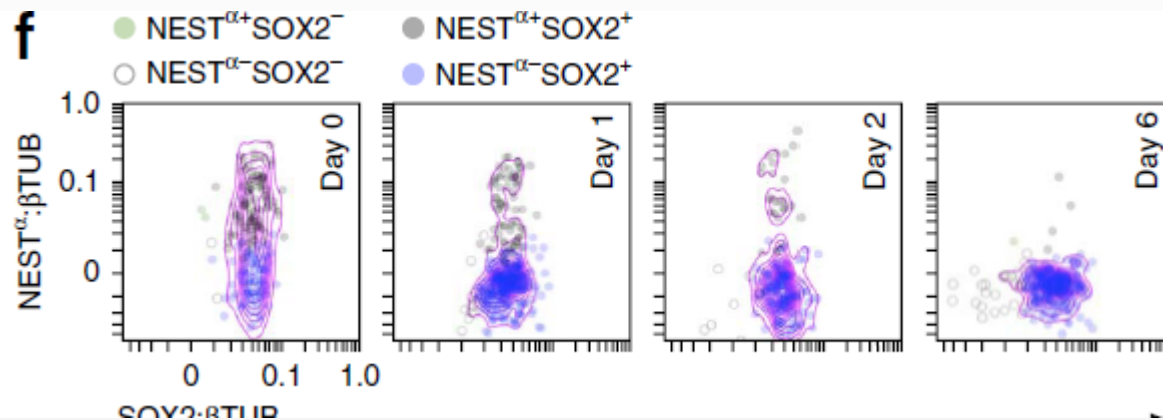
### Immunocytochemistry (ICC)





# Heterogeneity in NSC differentiation





NEST also exhibited two bands in conventional western blotting (114 and 270 kDa), **but** the extensive cell-to-cell variation in NEST<sup>α</sup> expression was not detectable with a conventional western

## CONCLUSIONS

- scWestern is a single-cell protein analysis technique capable of quantitative, multiplexed and at-the-bench operation
- offering an avenue to advance understanding of cell-to-cell variation in protein-mediated cell functions
- brings the specificity of a conventional western blot to the analysis of single cells, relevant in the context of heterogeneity in stem cell signaling trajectories
- by reporting molecular mass as well as antibody binding, the scWestern could identify two putative isoforms of a gene

## OUTLOOK

scWesterns – applications:

- that integrate upstream functional or morphological screens,
- quantify cell-to-cell response to pharmaceutical agents (including rare circulating tumor cells)
- affinity-reagent performance by easing library screens



**THANK YOU  
FOR  
YOUR  
ATTENTION!!!**

