



Single cell picking and multianalyte profiling

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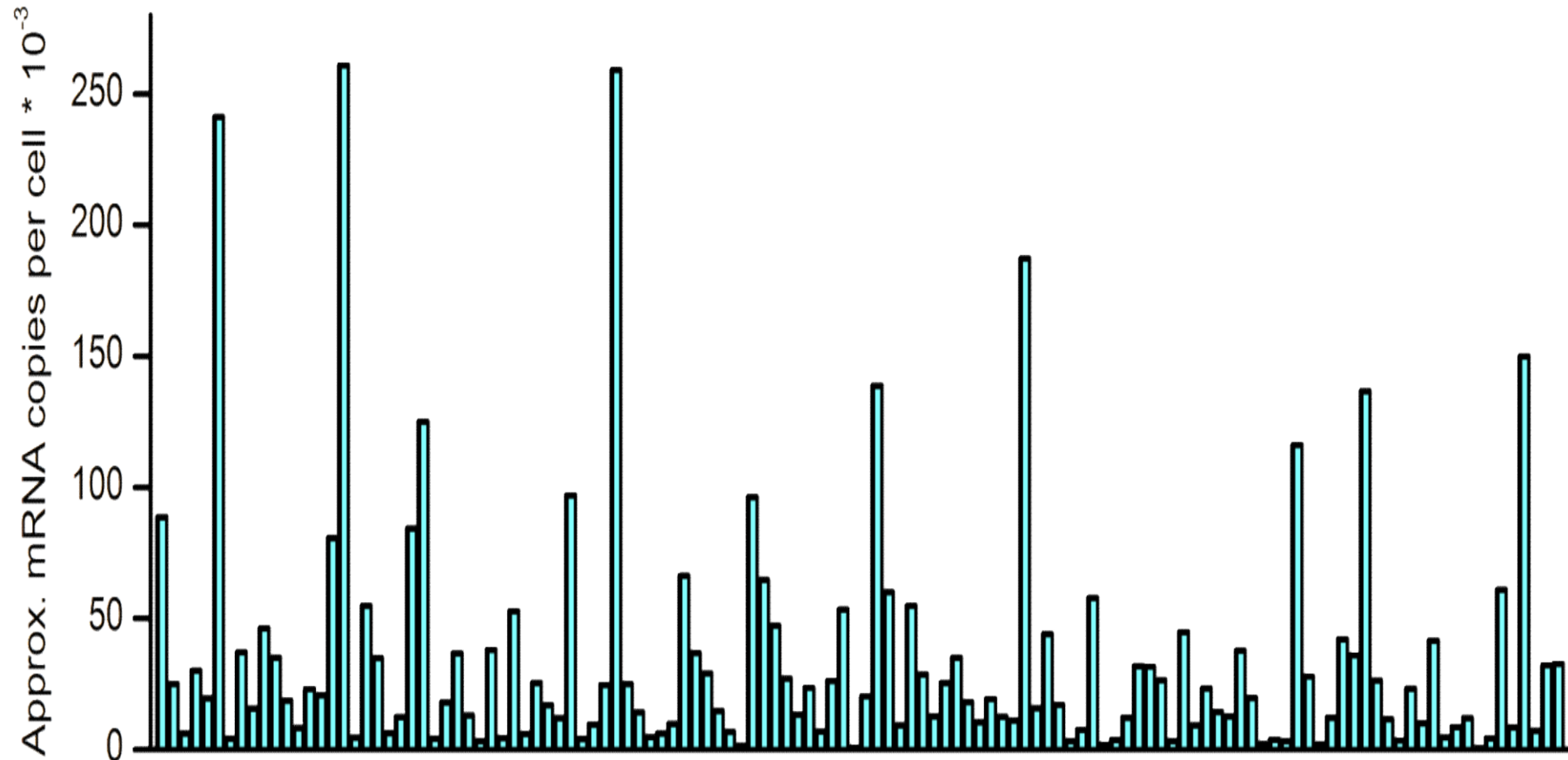
16.9.2016



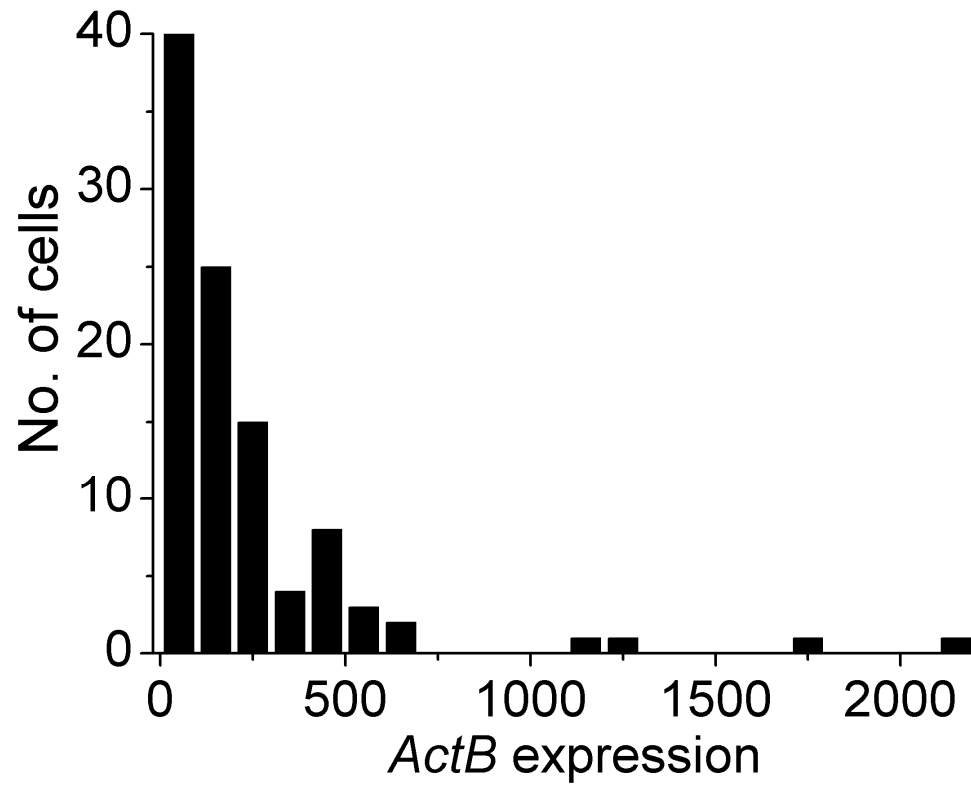
Why single-cell analysis?

- Cell population heterogeneity
- Identification and characterization of rare cells / subpopulations
- Discover and refined biomarker analysis

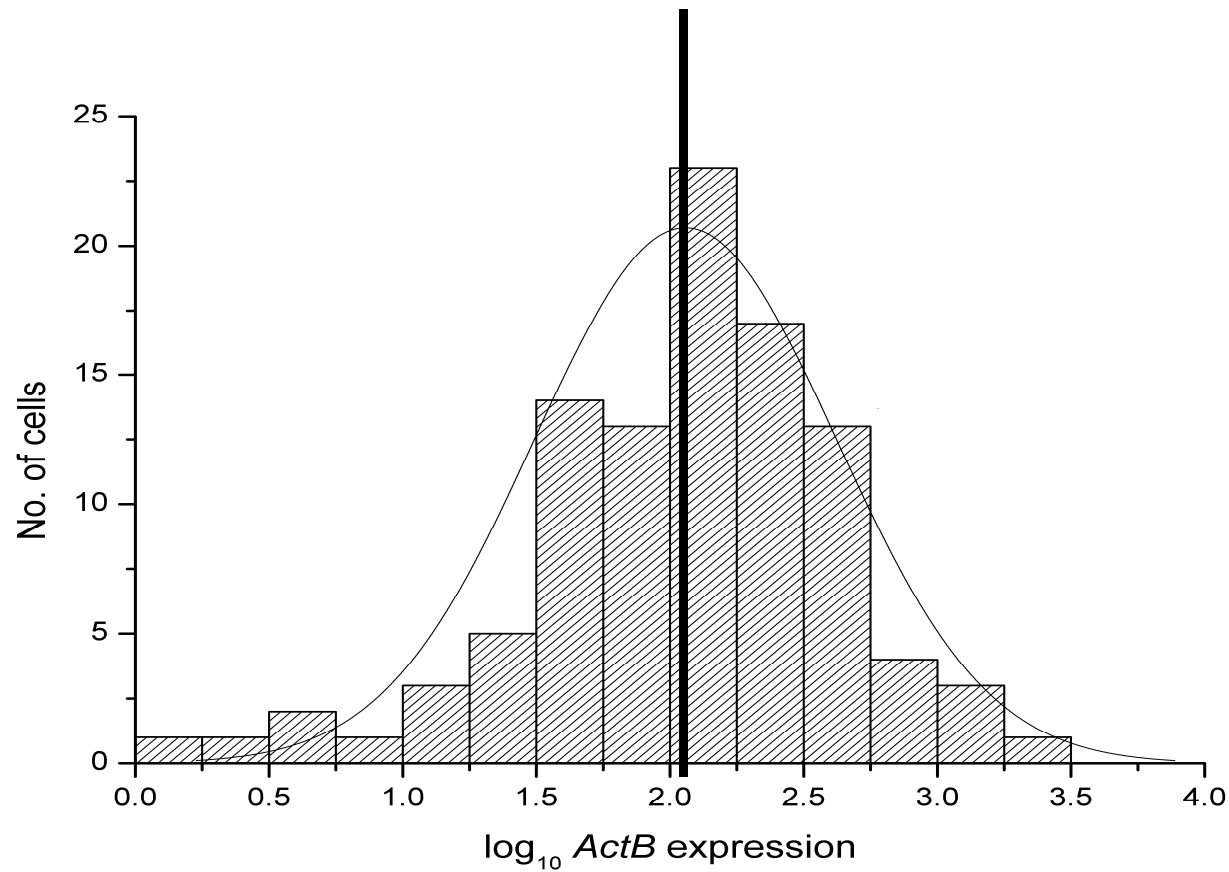
Typical single cell data – mRNA in 125 cells



Skewed distribution in linear scale



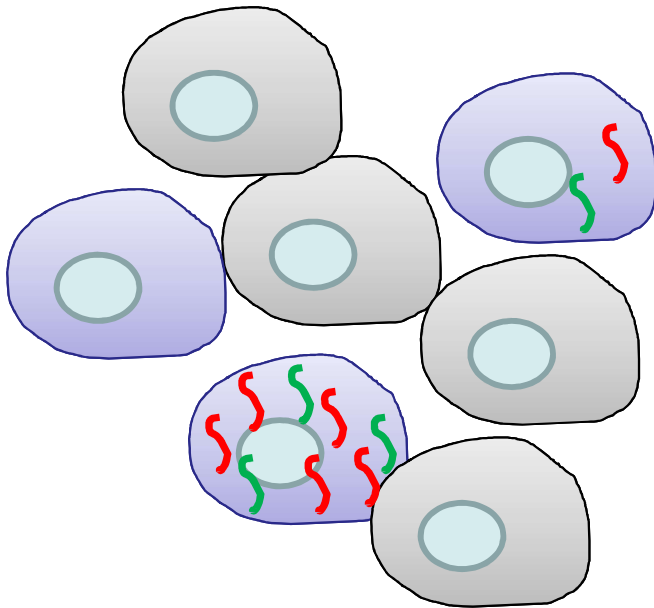
normal in logarithmic scale....



Interpretation of single-cell correlations

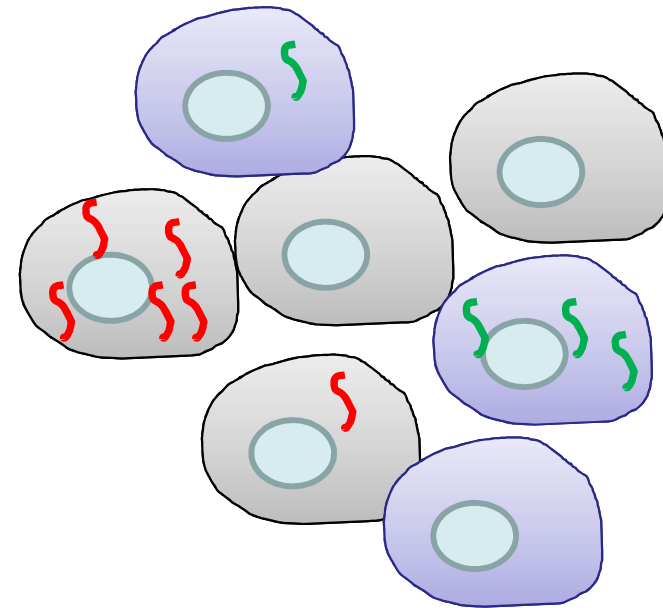
Total # of $\textcolor{red}{\text{S}}$ = 6

Total # of $\textcolor{green}{\text{S}}$ = 4

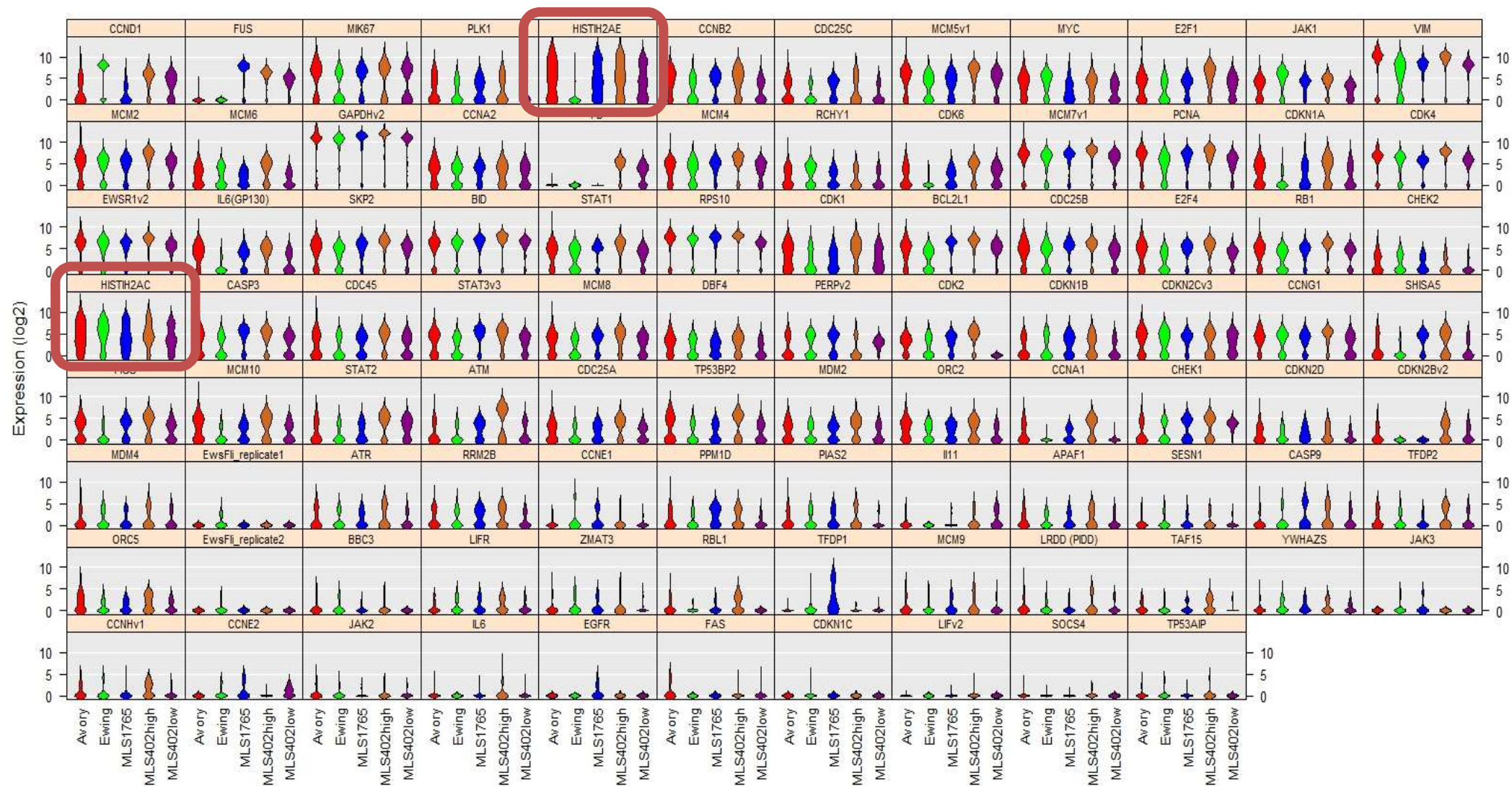


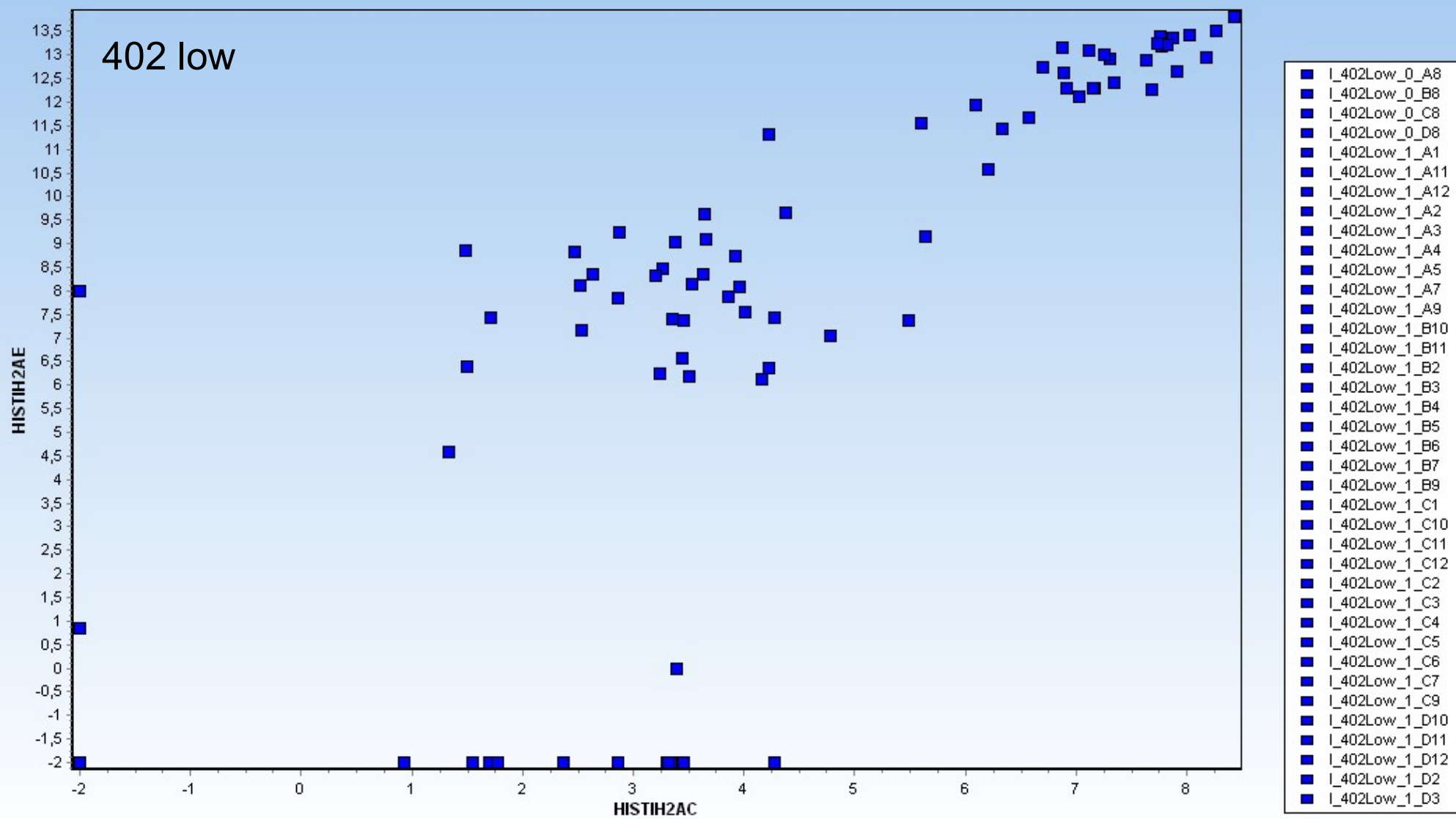
Total # of $\textcolor{red}{\text{S}}$ = 6

Total # of $\textcolor{green}{\text{S}}$ = 4

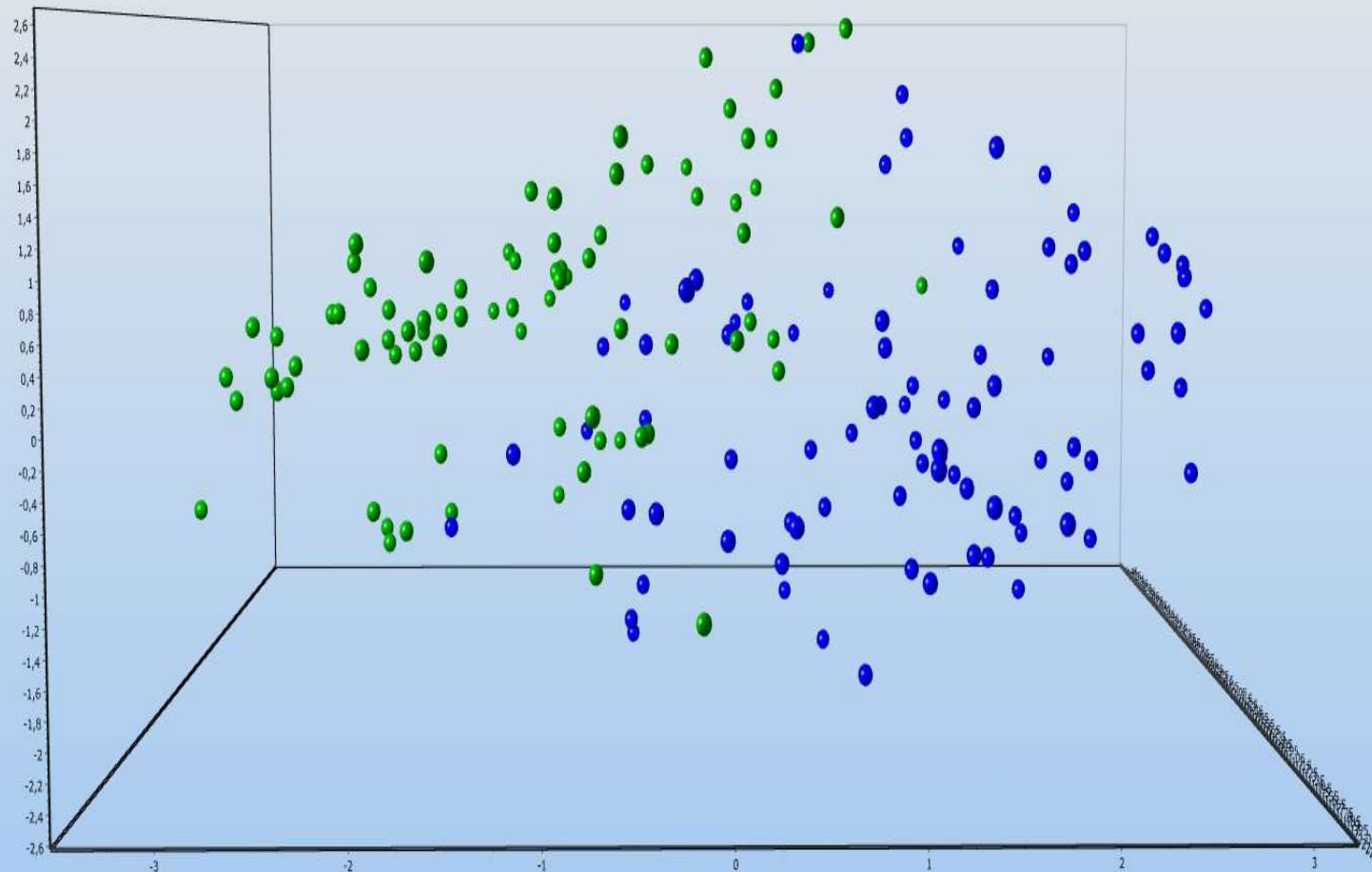


Violin Plot of Gene Expression





Single cell data: MLS402 low serum, high serum



involved genes: CCNA1, DBF4, IL6, CCNE2, PERPv2, CCNHv1, MCM8



Fully automated platform for isolation of **single cells**, cell **clusters** and cell **colonies**



Ultra-precise robotics
and pipetting tools
for mechanical
cell isolation

Automated
high resolution
fluorescent
microscope

Powerful image analysis
software

▲ Unique combination of image recognition, image processing, cell harvesting and documentation

Culture well – overview
Prostate cancer patient

5 mm

Culture well – overview

Prostate cancer patient

17

18

19

20

21

22

23

24

25

19 - Before

Source: 160518_CTC z Karlaku_zbyle 3 jamky | A-1

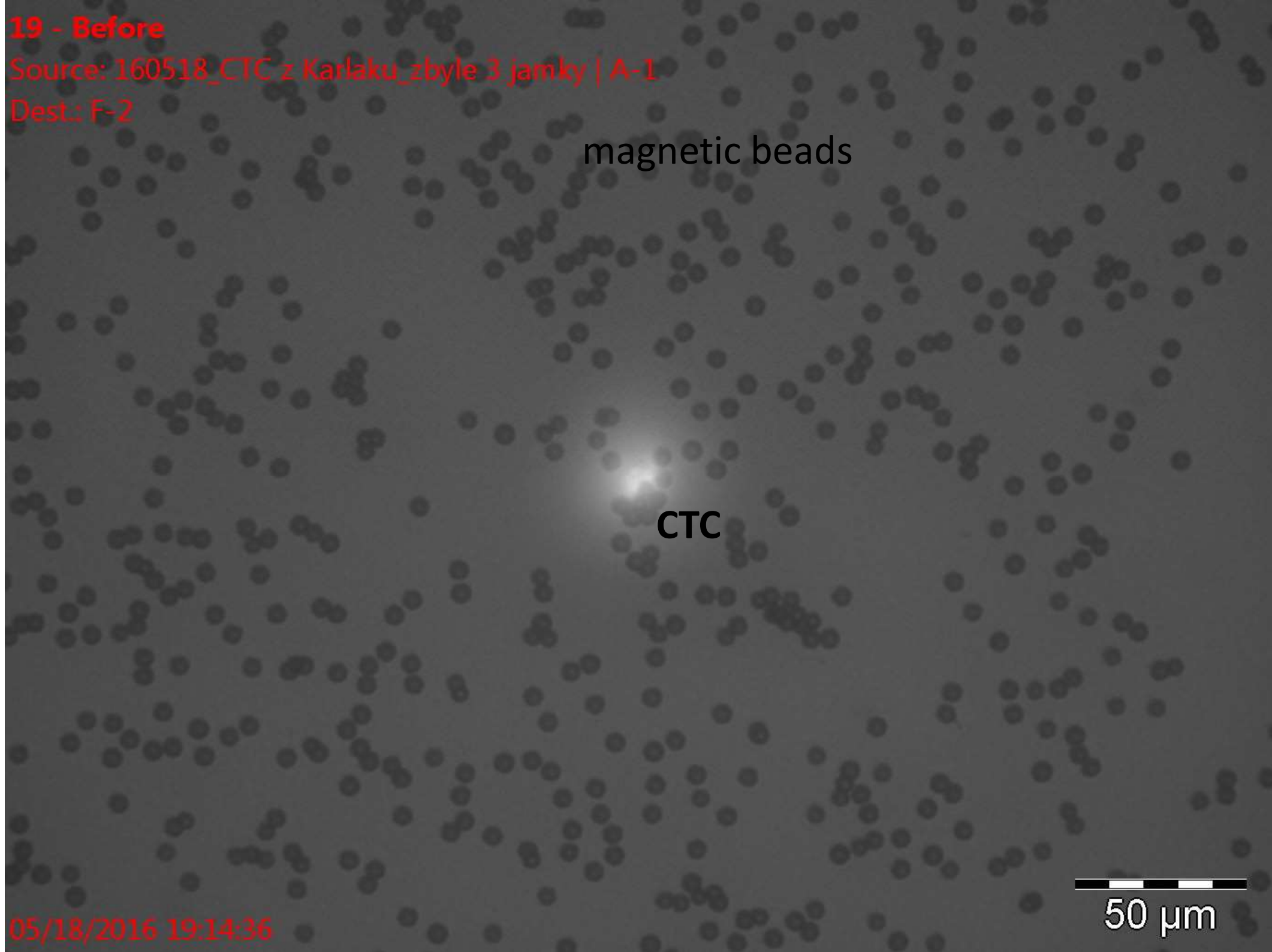
Dest.: F-2

magnetic beads

CTC

05/18/2016 19:14:36

50 μm



19 - After

Source: 160518_CTC z Karlaku_zbyle 3 jamky | A-1

Dest.: F-2

Stain:

- nucleus (shape, size)
 - epithelial: **EpCAM**, E-CAD, MUC, (CKs)
 - mezenchymal: (VIM)
 - EMT: **E-CAD**, N-CAD, (VIM)
 - WBC: **CD45**, ...
-
- characterize using CellCelector into groups
 - single cell profiling (BioMark)



8 - Before

Source: 160211_Katarina CTC_manual_CF open day | A-1

Dest.: G-1

Filter membrane

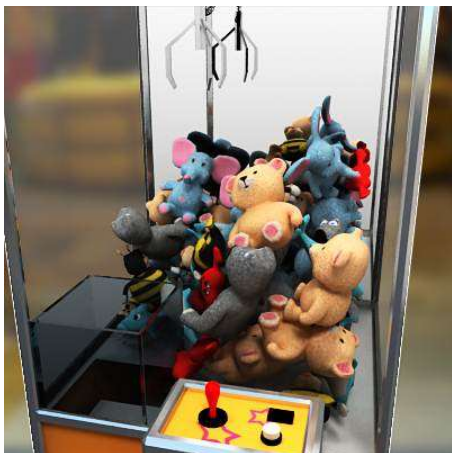
CTC

02/11/2016 15:12:51

100 μm



When we started..



?

Attached cells



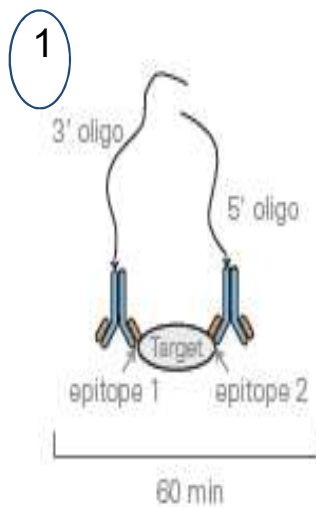
60–80 %
(+ potential background)

Sedimented cells



80–100 %

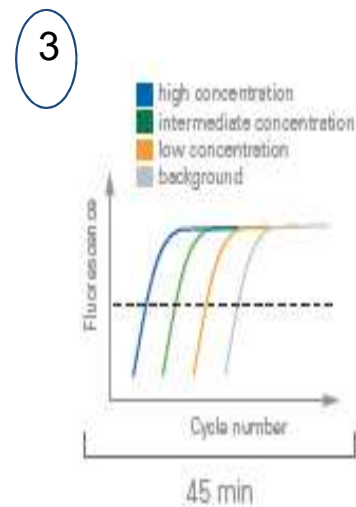
Proximity Ligation Assay (PLA)



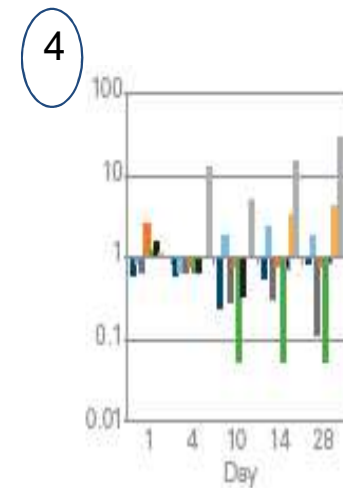
Binding of Probes to Protein Targets



Ligation of Oligos in Proximity

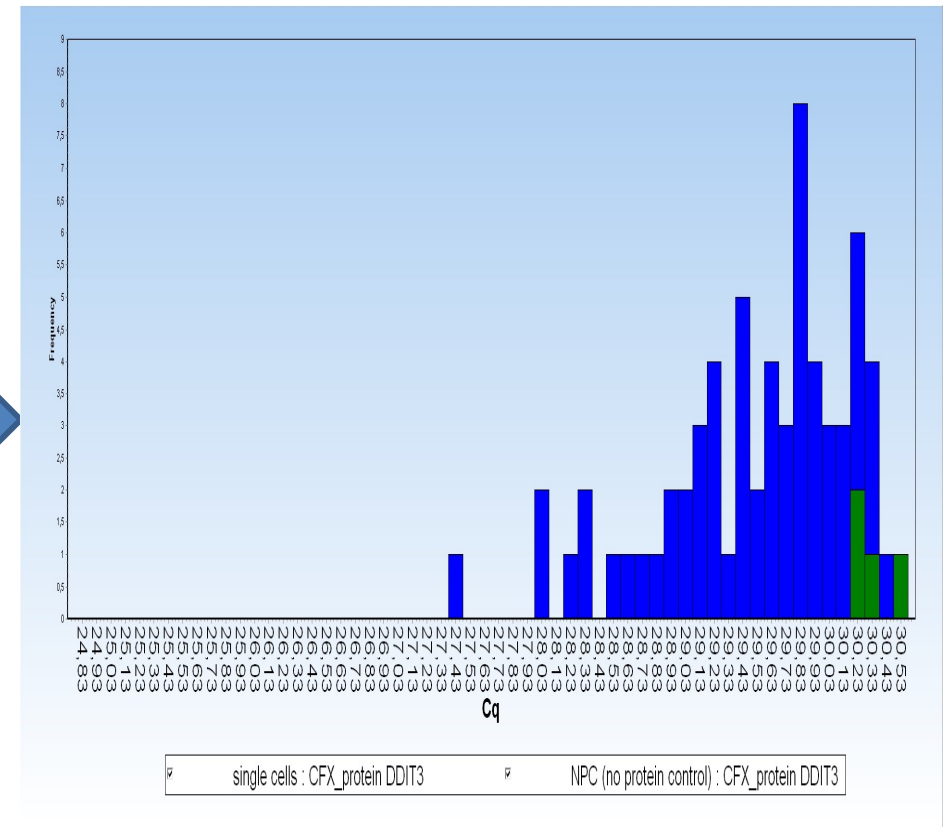
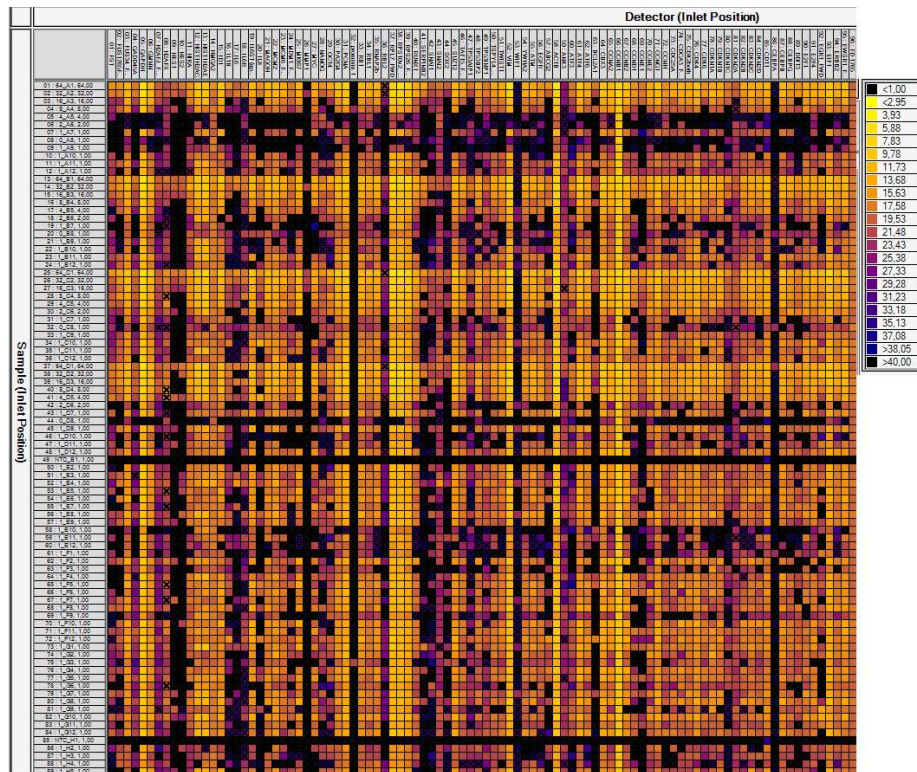


Real-time PCR



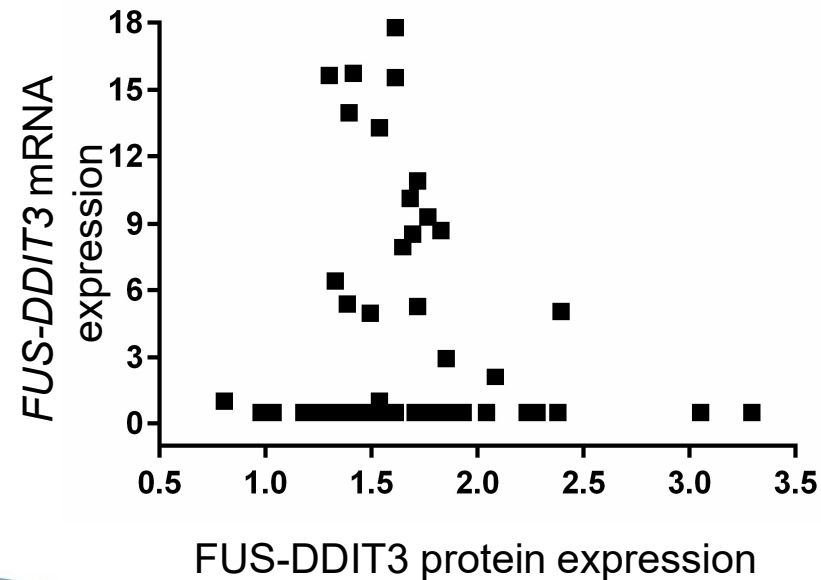
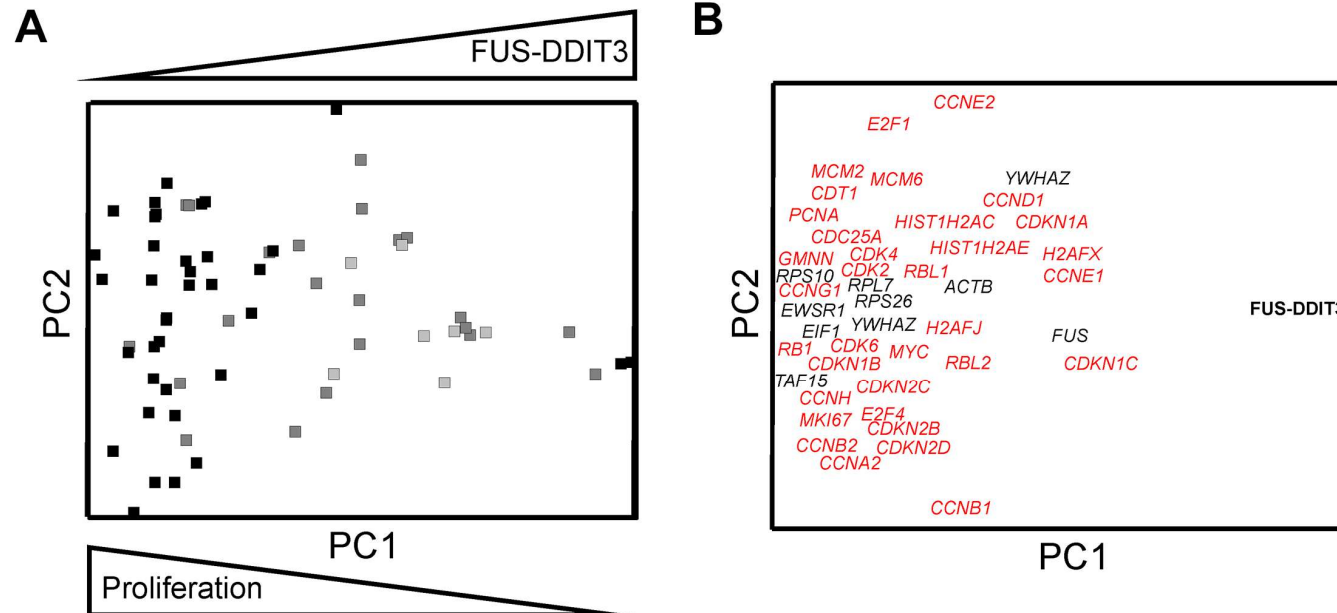
RQ Data Analysis

Proximity Ligation assay for FUS-DDIT3: single cells MLS 1765

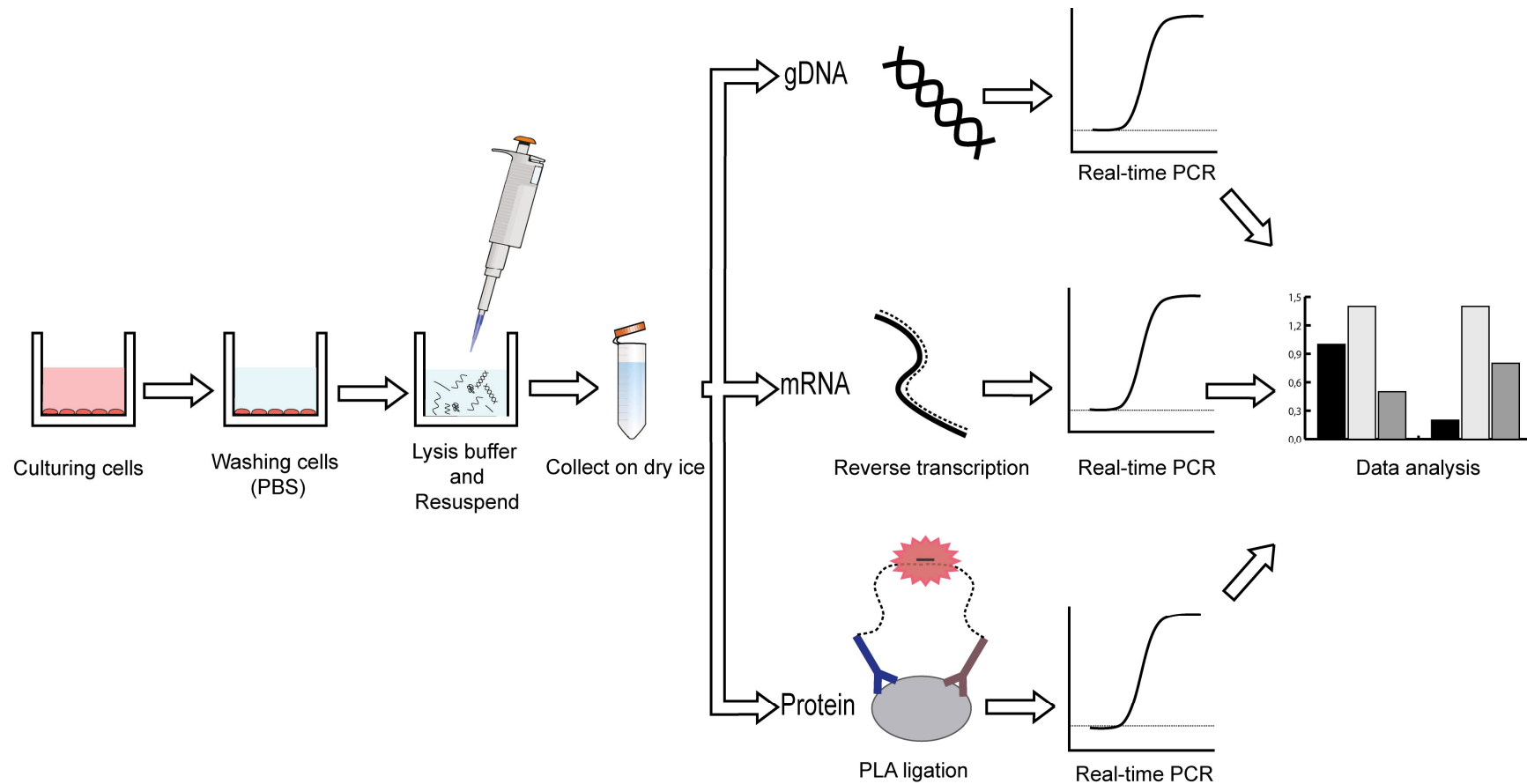


Combination and mRNA and Protein analysis of individual cells

—— Association of FUS-DDIT3 expression and cell proliferation ——

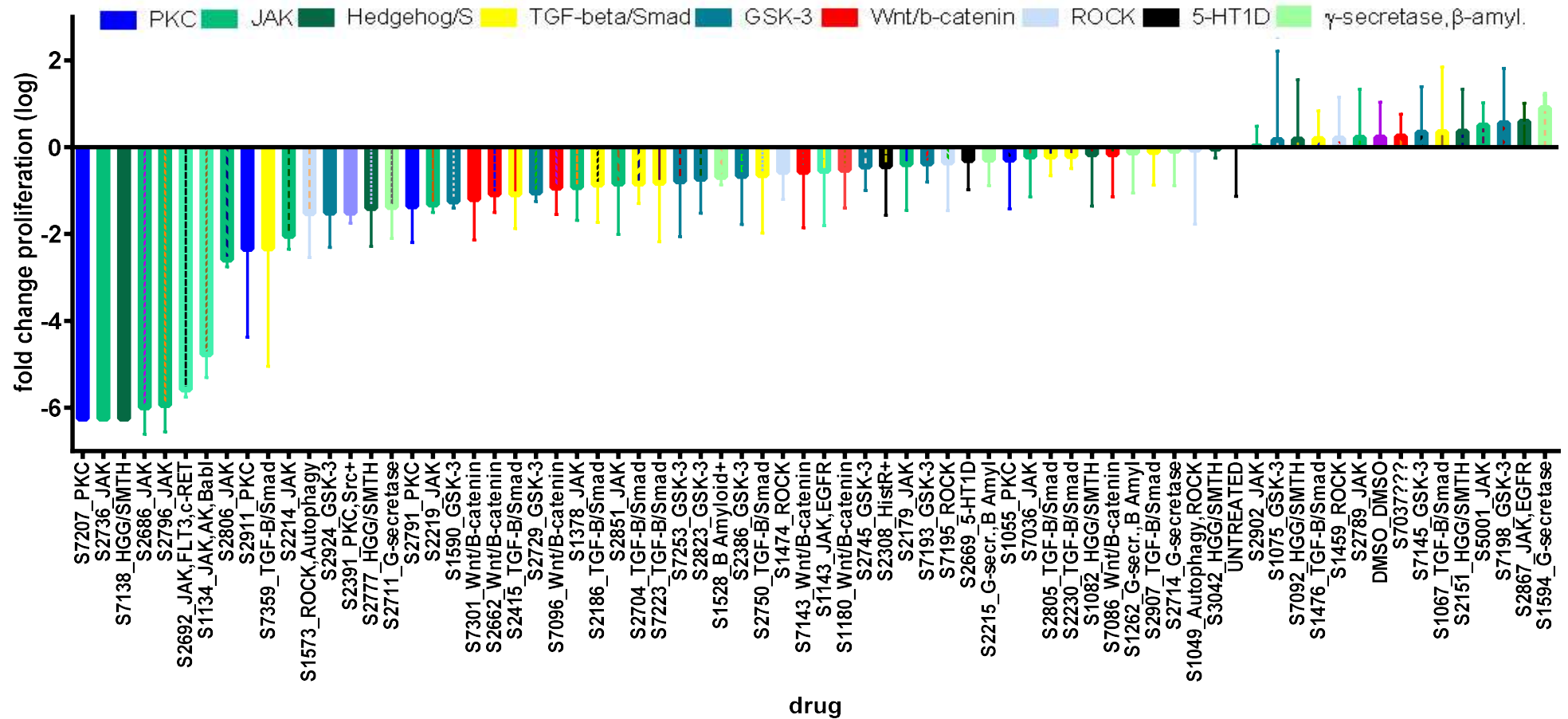


Workflow: RT-qPCR bulk analysis



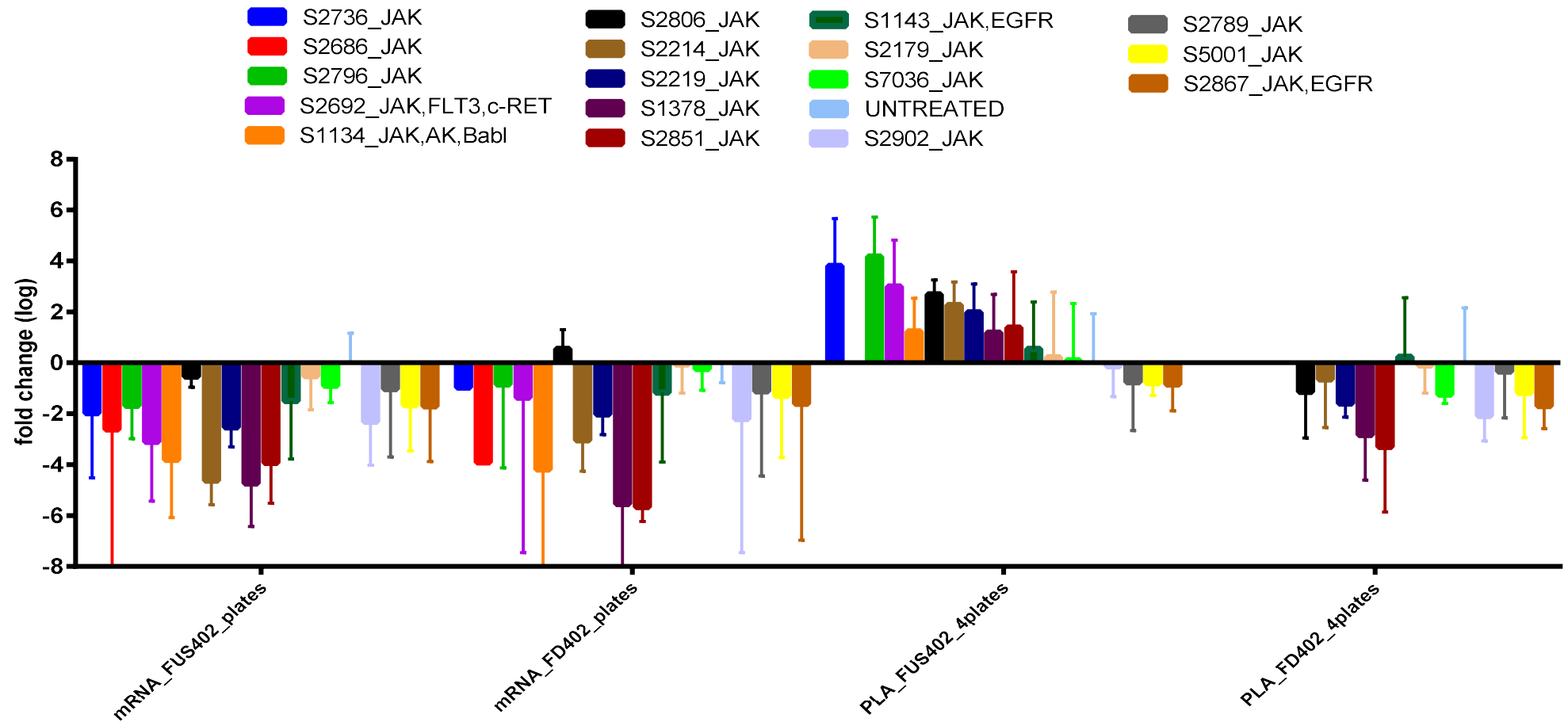
80 drugs applied to ~10 000 cells of MLS 402, incubated for 72h

gDNA 1-4 averaged (4 biol.replicates)



80 drugs

JAK-STAT targets





- **Prague:**

Radek Šindelka, Pavel Abaffy (SK)

Lukáš Valihrach, Daniel Žucha, Peter Androvič (SK)

Ravindra Naraine (Karibik), Ondřej Smolík, Silvie

Tománková, Veronika Kašparová, Mikael Kubista

Veronika Mikulová, Katarina Kološtová (SK)

Jiří Neužil, Martina Bajzíková, Jaromíra Kovářová

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Vlasta Korenková, Lucie Langerová (Core Facility)

Petra Grznárová, Z. Cimbůrek (FACS)

- **Gothenburg:**

Anders Ståhlberg (S)

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Tom Kroneis (AUT)

Paul Fitzpatrick (IRL)

Nina Akrap (D)

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Pernila Grundevik (S)

Pernila Gregerson (S)

Göran Landberg (S)

Mikael Kubista (CZ/S)