

**Why the expression levels of some known drug target genes are not regulated by the drug?  
(expression: balance between transcription & degradation)**

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**BRIC KØBENHAVNS UNIVERSITET**



# Conflict of interest disclosure

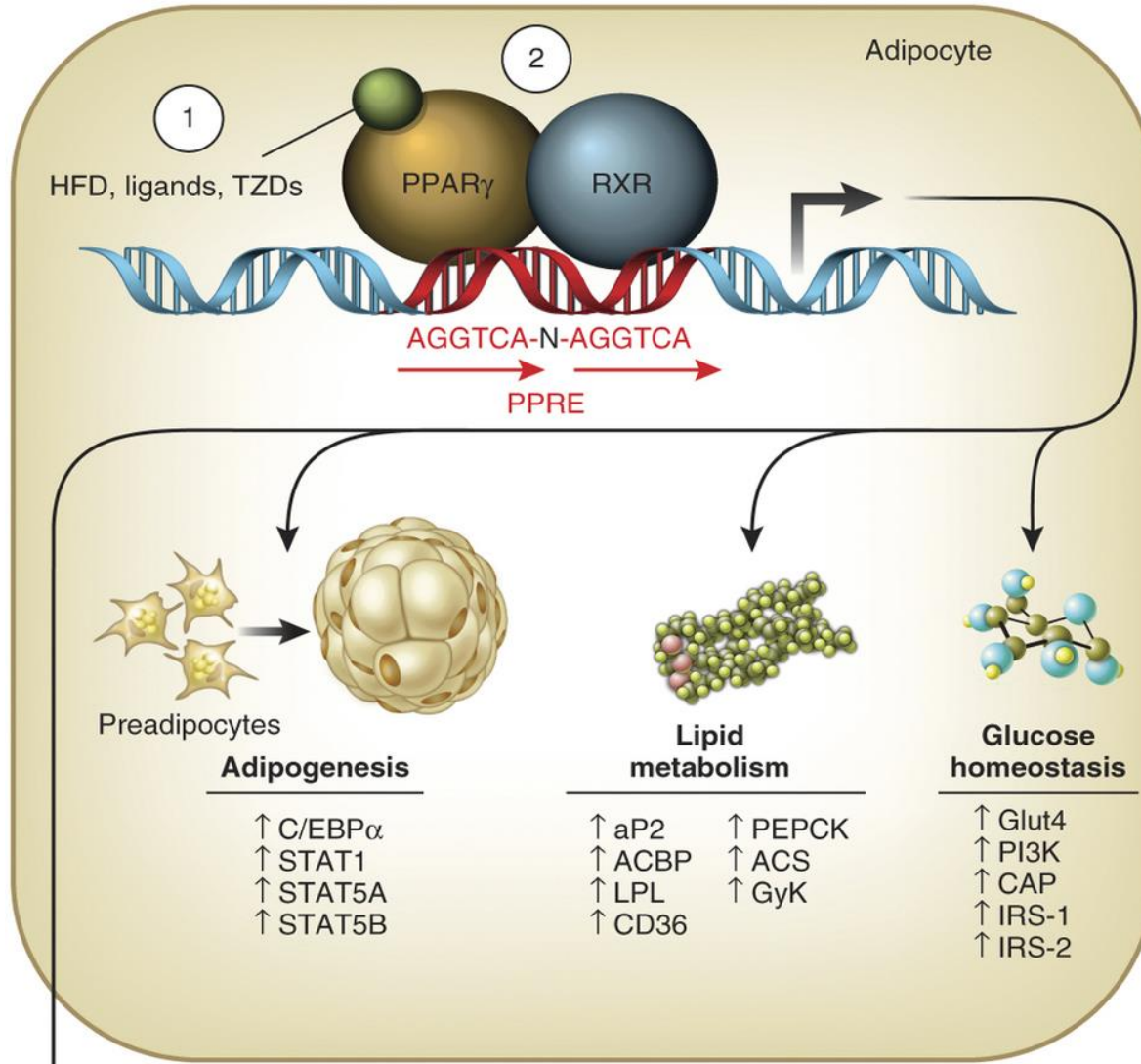
**None**

**Committee of Scientific Affairs**



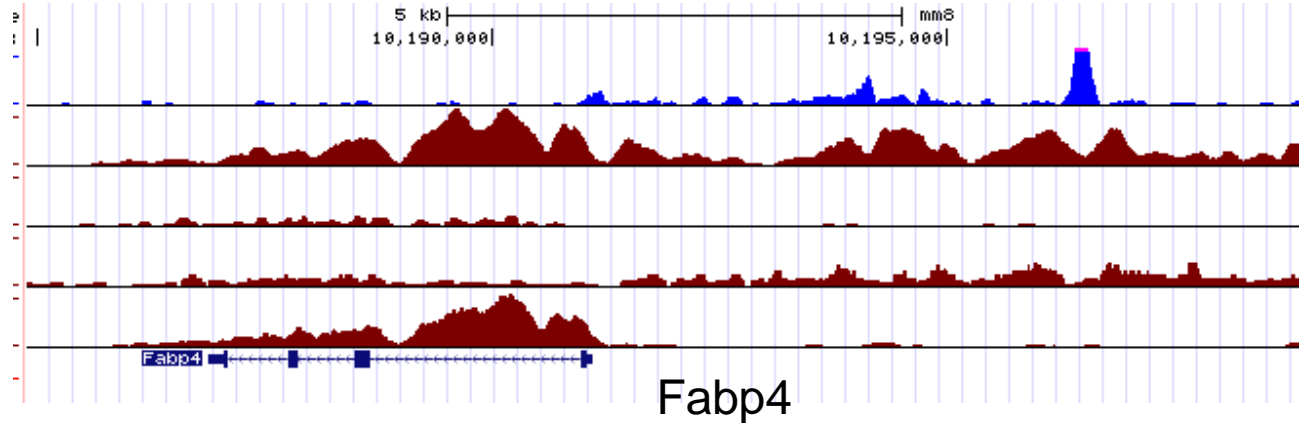
**Committee of Scientific Affairs**

# Anti-diabetic rosiglitazone (Rosi) is an agonist of PPAR $\gamma$

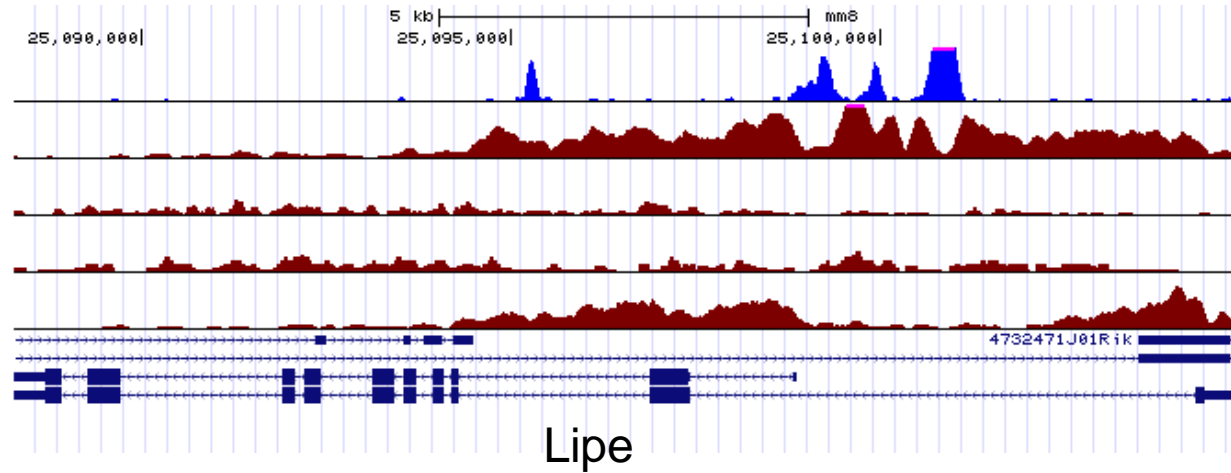


# Identifying PPAR $\gamma$ target genes using ChIP-seq

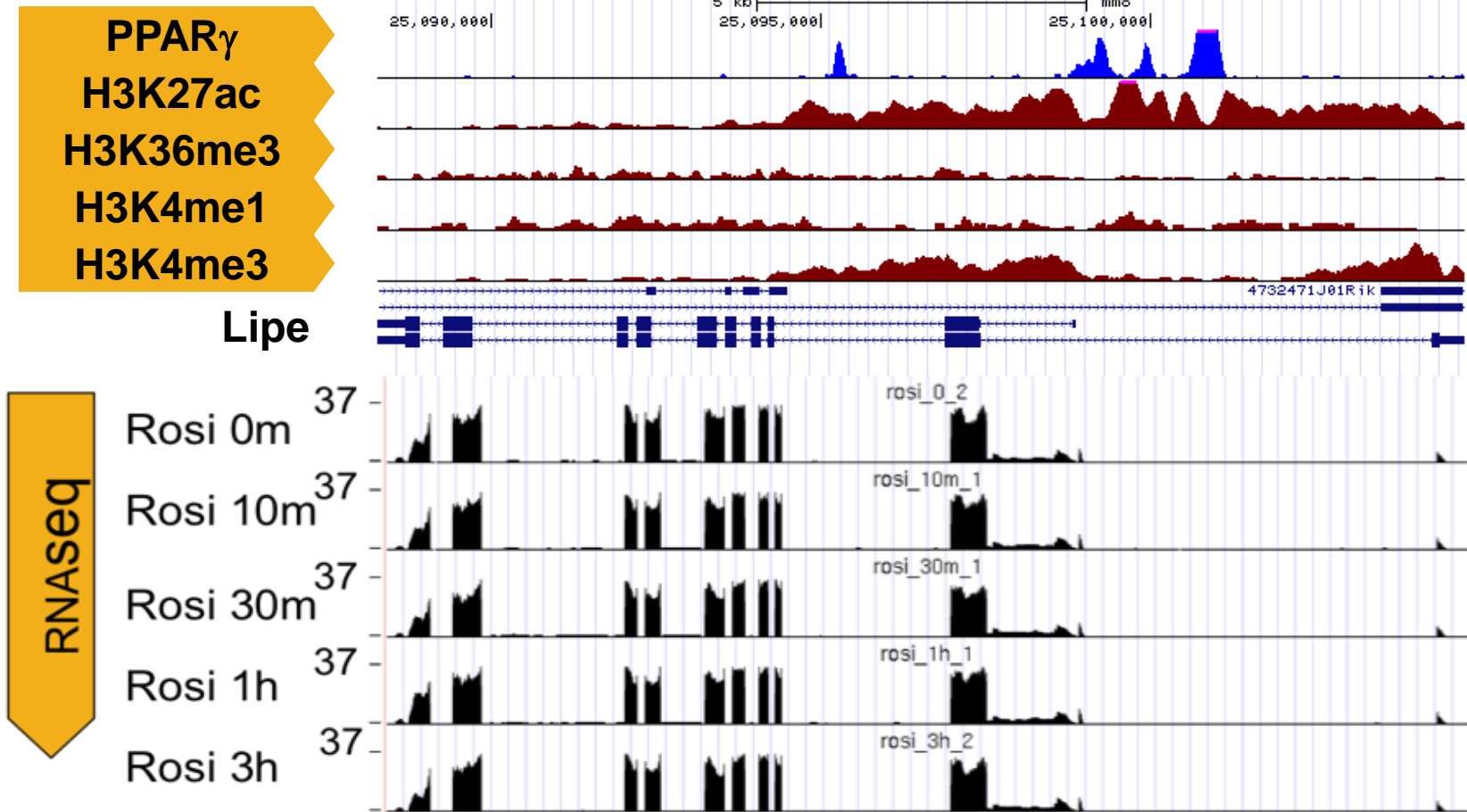
PPAR $\gamma$   
H3K27ac  
H3K36me3  
H3K4me1  
H3K4me3



PPAR $\gamma$   
H3K27ac  
H3K36me3  
H3K4me1  
H3K4me3



# Gene expression levels (RNAseq) were not changed by rosi for Lipe



## Why?

1. *Lipe* is not a target of PPAR $\gamma$ . But log<sub>2</sub>FC after PPAR $\gamma$  KD: -3.2
2. *Lipe* is upregulated but post-transcriptionally down regulated

# The balance between mRNA synthesis and decay determines mRNA expression levels

Transcription  
(mRNA synthesis)

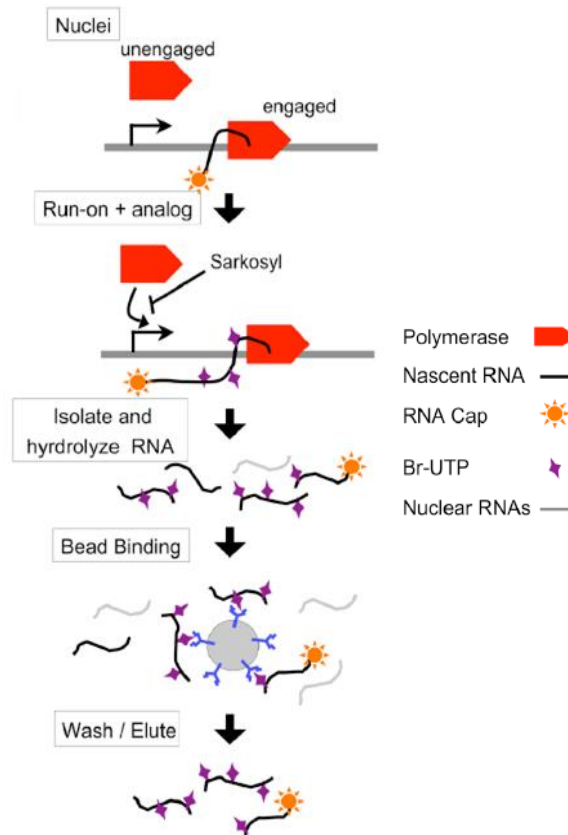


Gene expression



Degradation

GROseq



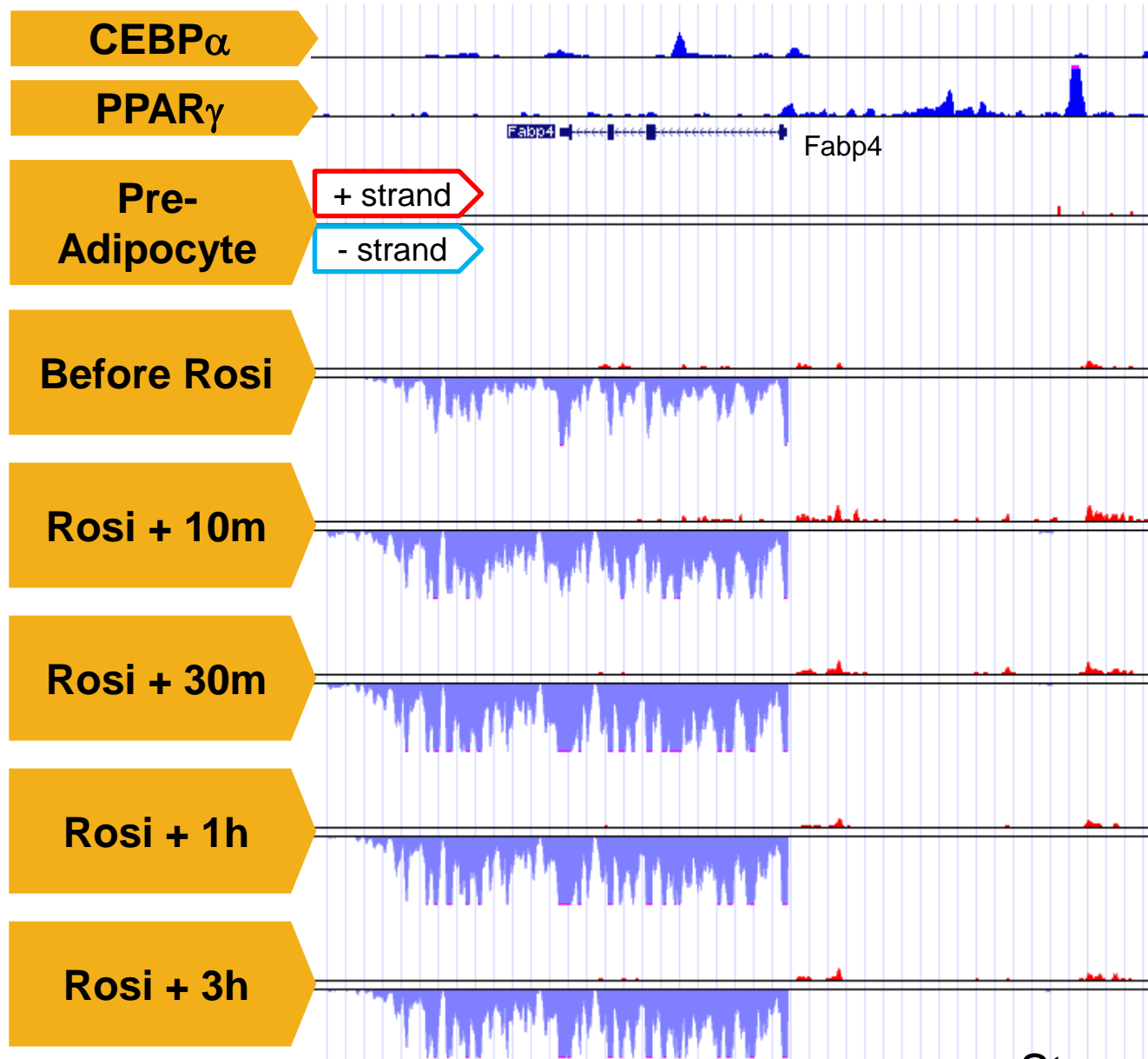
RNA-seq  
microarray

?

## GRO-seq

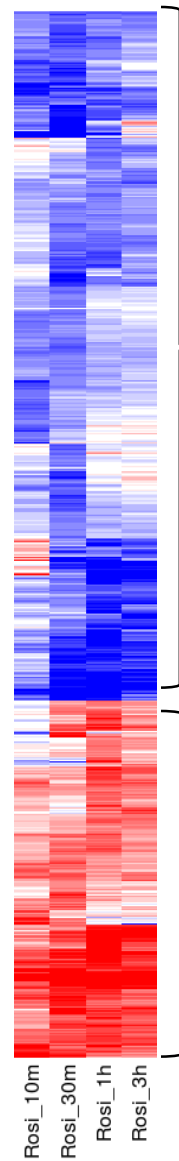
- Global Run On – sequencing
- Isolate all newly synthesized RNAs in a cell
- Measures active transcription rather than steady state mRNA levels
- Maps the position and orientation of new transcripts
- Earliest changes can identify primary target genes rather than secondary targets
- Unbiased detection of novel transcripts, including non-coding RNAs
- detects enhancer RNAs

# Up Regulation of Transcription Due to Rosi



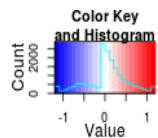
# Up and Down Regulation of Transcription Due to Rosi

Log2-FoldChange  
in Gene Body

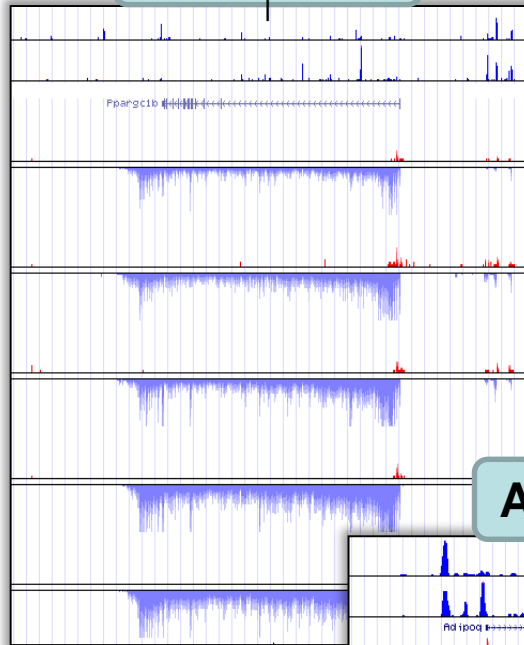


1384

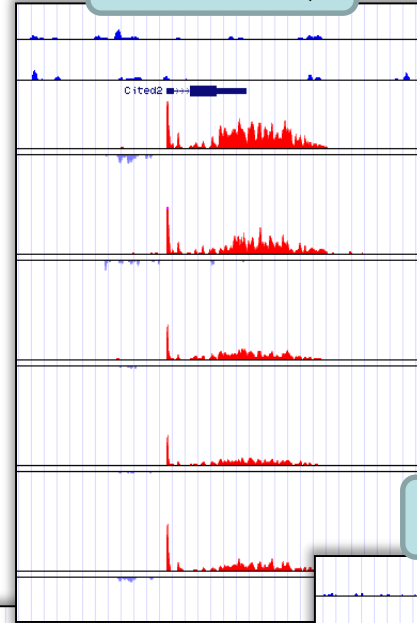
567



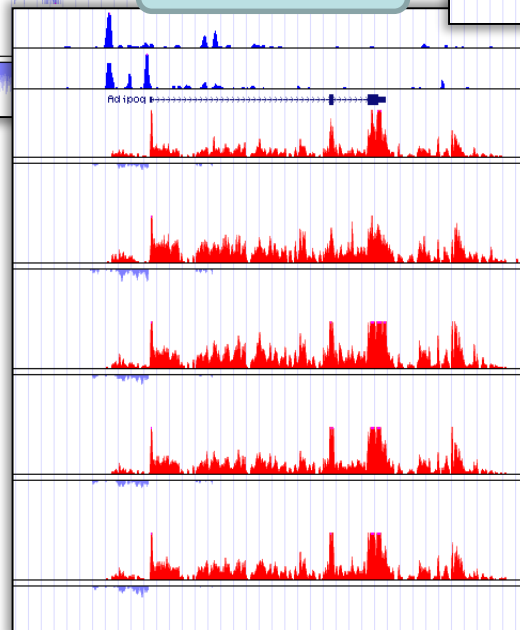
Ppargc1b: ↑



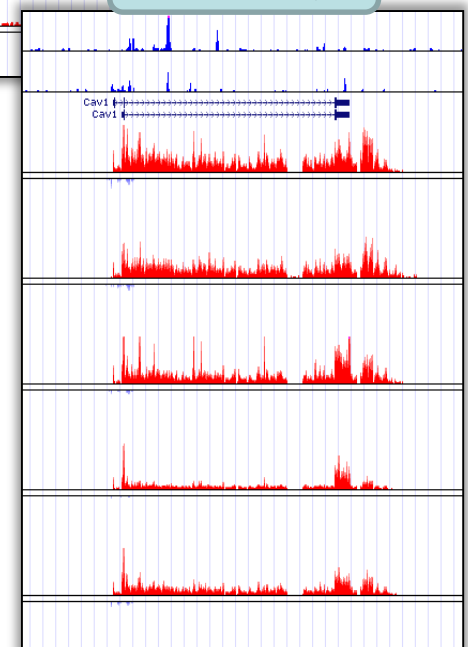
Cited2: ↓



Adipoq: ↑

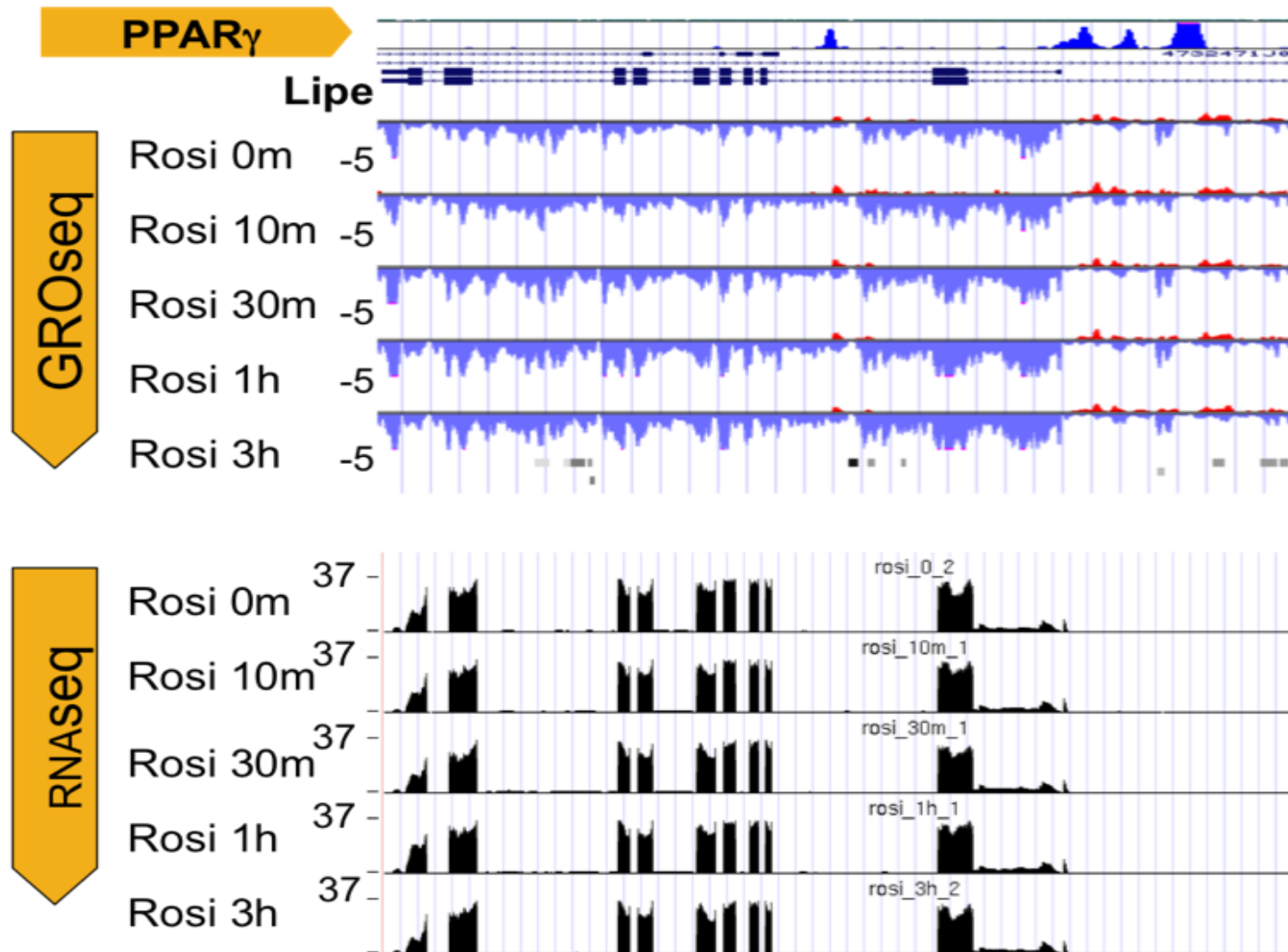


Cav1: ↓



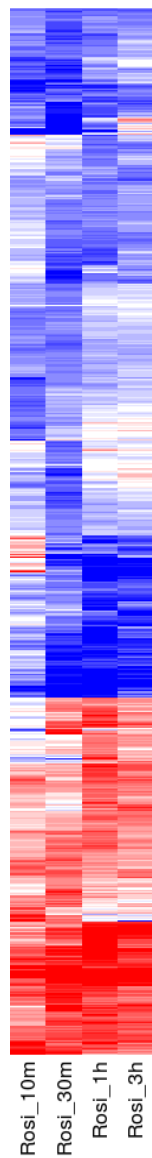


# Gene expression levels (RNAseq) were not changed by rosi but the transcriptional levels were changed for Lipe

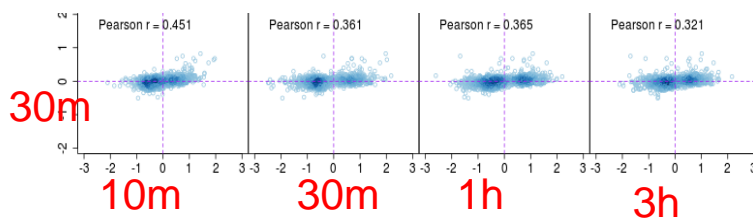
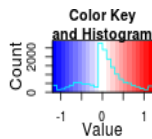


# Nascent Transcription Changes Precedes mRNA Regulation

Log2-FoldChange  
in Gene Body



Steady-state mRNA

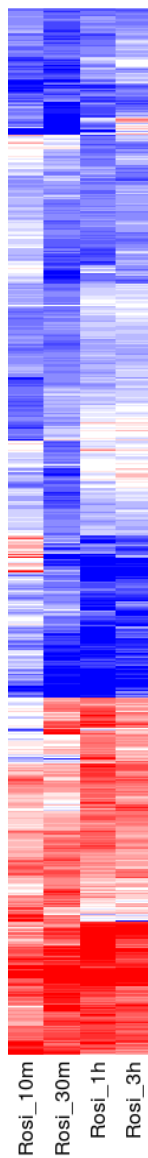


GROseq

Step et al. (2014) G&D

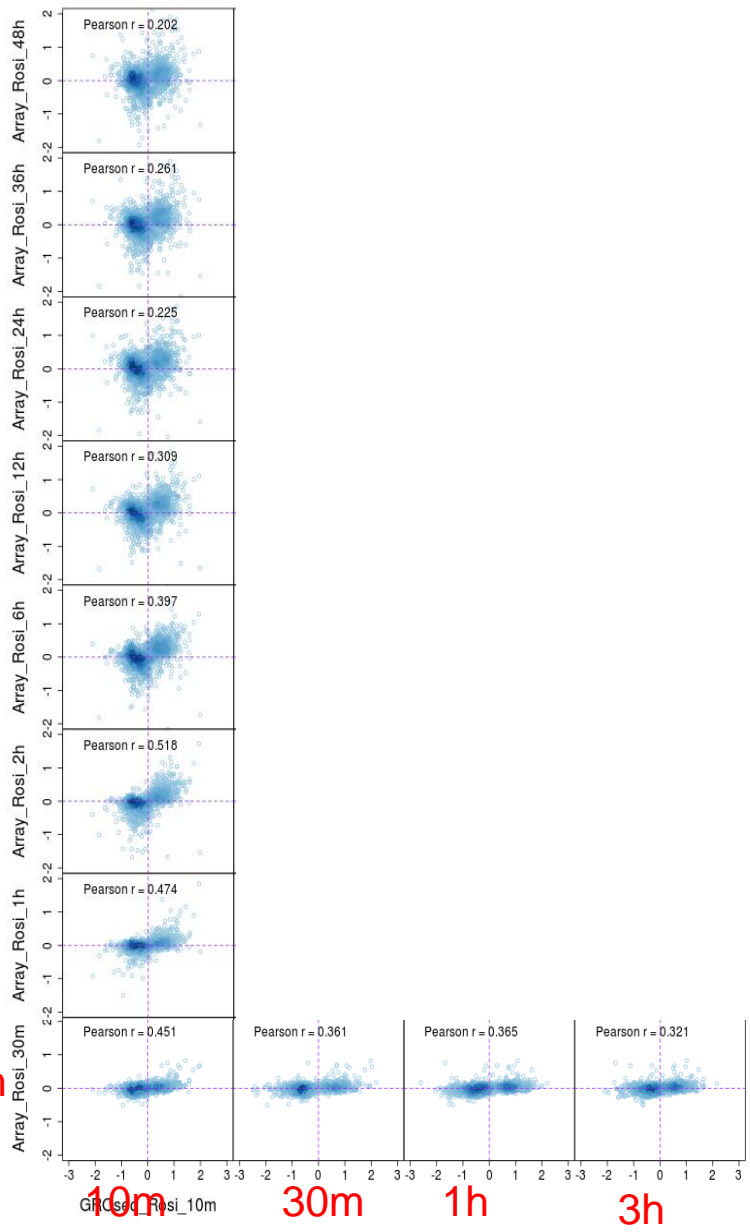
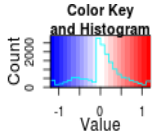
# Nascent Transcription Changes Precedes mRNA Regulation

Log2-FoldChange  
in Gene Body



Steady-state mRNA

48h  
36h  
24h  
12h  
6h  
2h  
1h  
30m

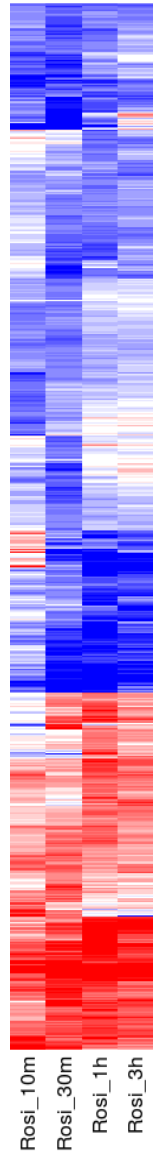


GROseq

Step et al. (2014) G&D

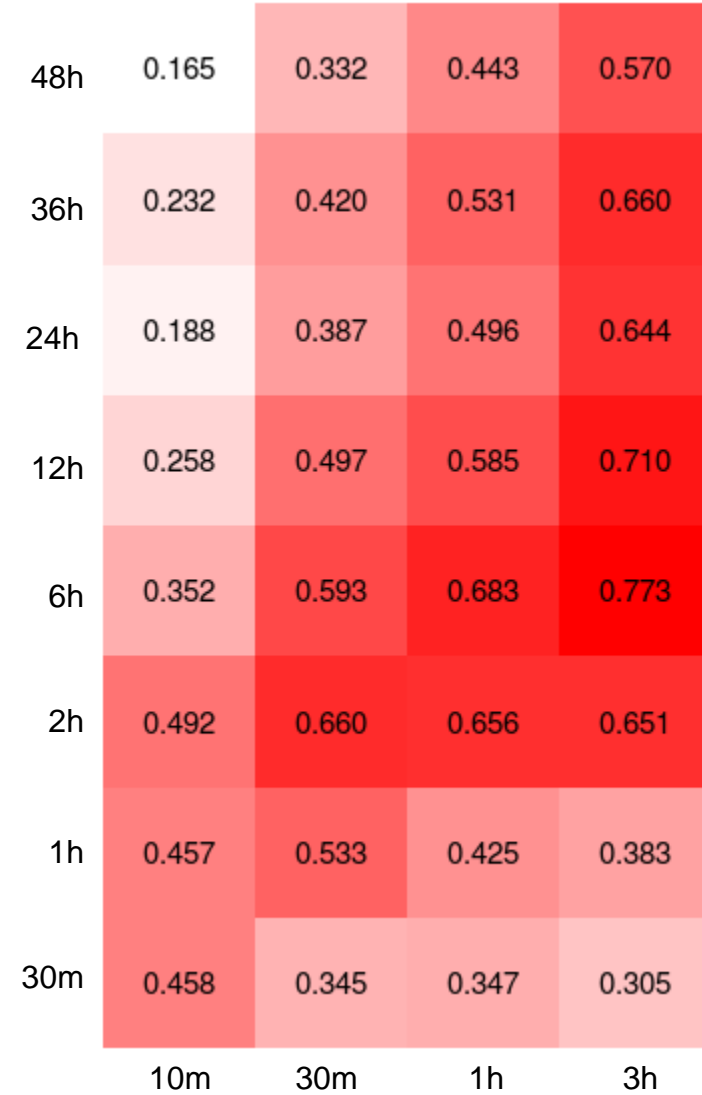
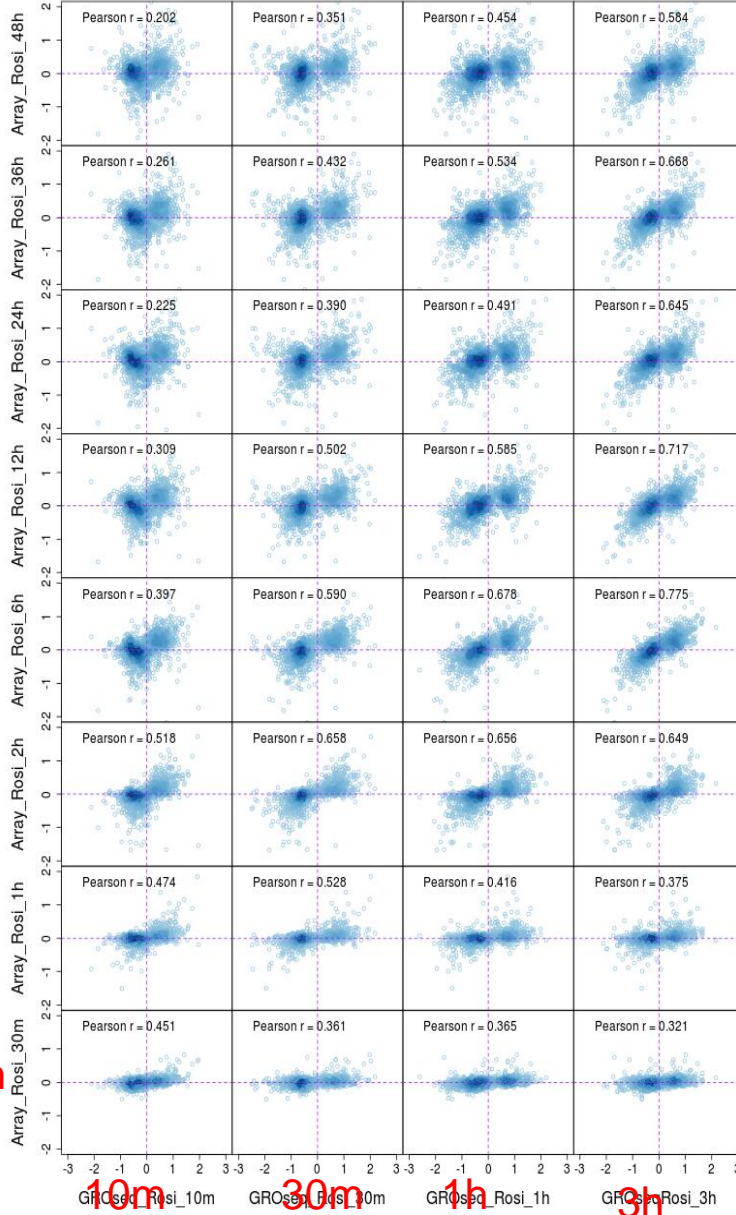
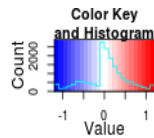
# Nascent Transcription Change Precedes mRNA Regulation

Log2-FoldChange  
in Gene Body



Steady-state mRNA

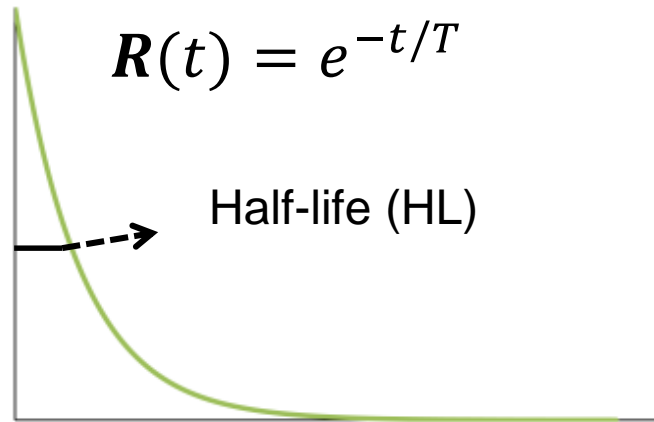
48h  
36h  
24h  
12h  
6h  
2h  
1h  
30m



Step et al. (2014) G&D

# Calculation of mRNA half-life using a dynamic model

When  $G(t)=0$



$$\frac{dR(t)}{dt} + \frac{R(t)}{T} = KG(t)$$

$G(t)$ : mRNA synthesis (GROseq)

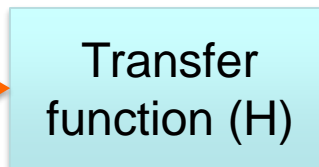
$R(t)$ : mRNA expression (RNAseq)

$T$ : time constant

$K$ : gain of the synthesis

Half-life :  $T \cdot \ln 2$

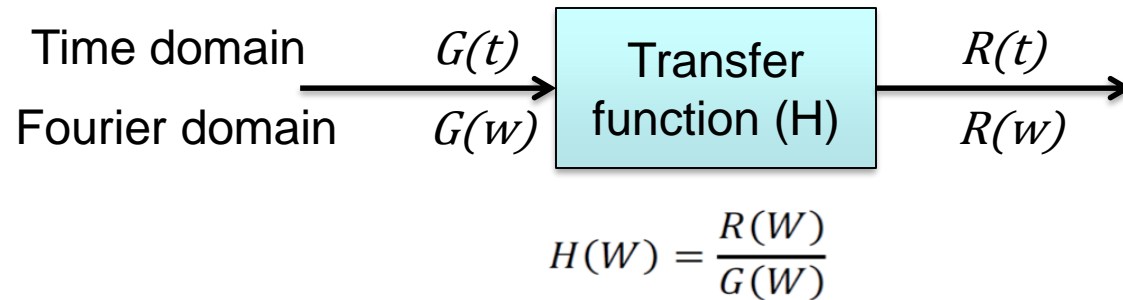
Transcription Level  
(GROseq;  $G(t)$ )



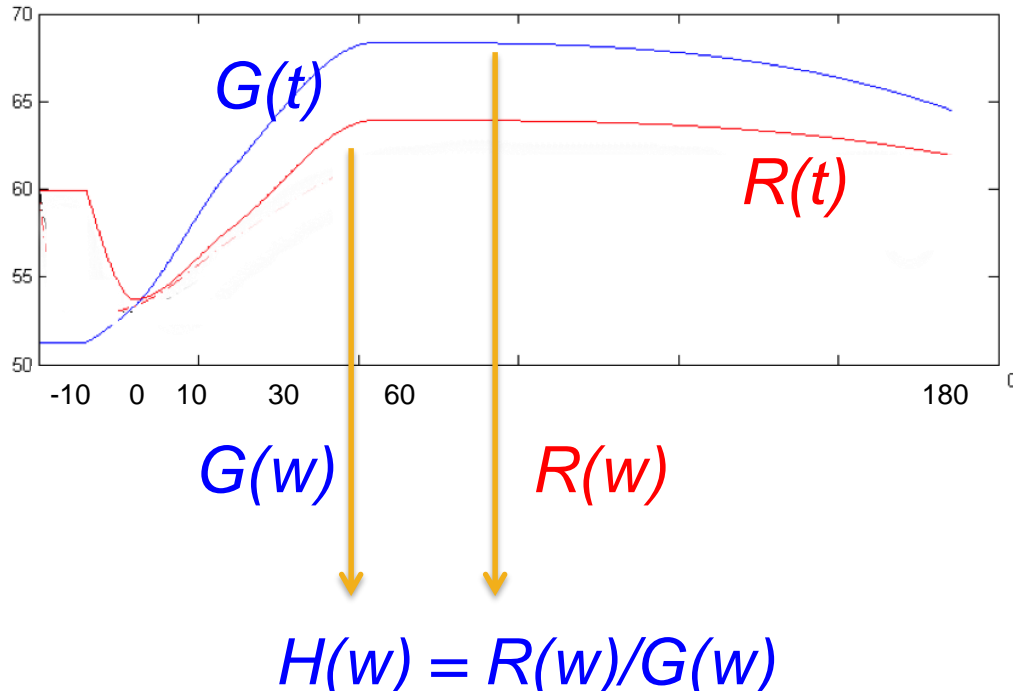
Expression Level  
(RNAseq;  $R(t)$ )

- GROseq (2 replicates)
  - t: 0, 10, 30, 60, 180 min after rosi treatment
- RNAseq (2 replicates)
  - t: 0, 10, 30, 60, 180 min after rosi treatment

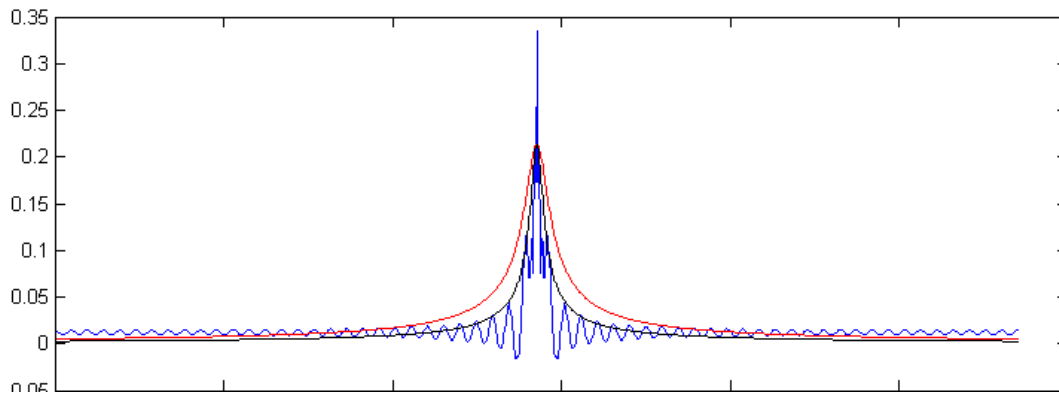
# Dynamic models for stability



# Modeling the signals in Fourier domain

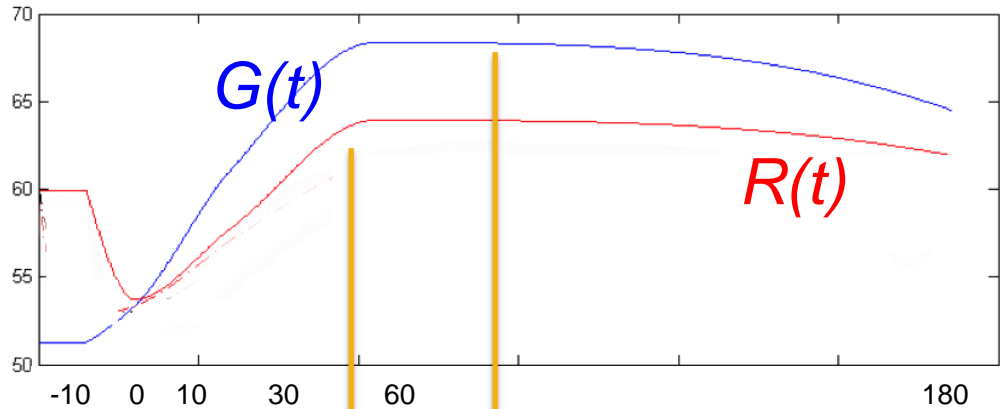


$$H(w) = R(w)/G(w)$$



Transfer function in  
the Fourier Domain

# Modeling the signals in Fourier domain



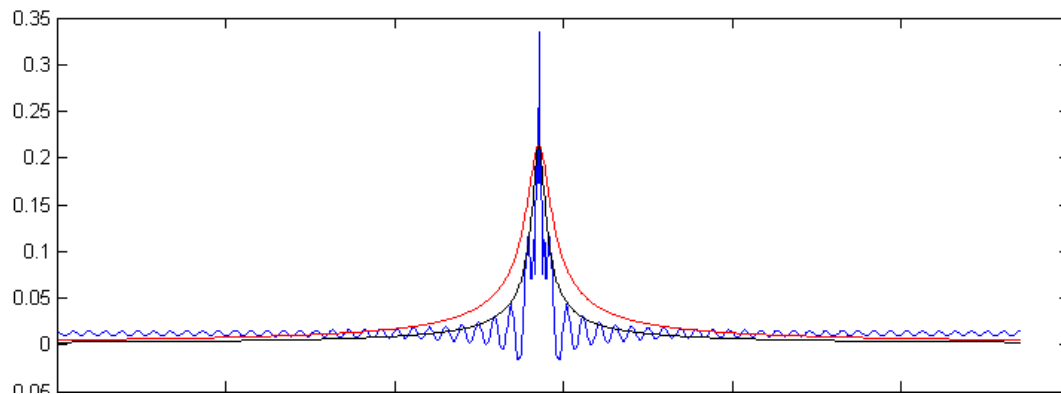
$G(w)$

$R(w)$

$$H(w) = R(w)/G(w)$$



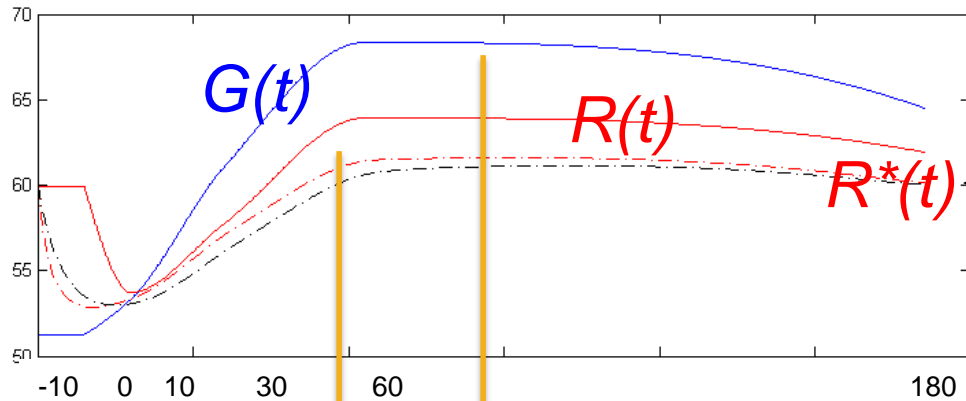
$H^*(w)$  or  $H^*(w)$



Transfer function in  
Fourier Domain



# Modeling the signals in Fourier domain



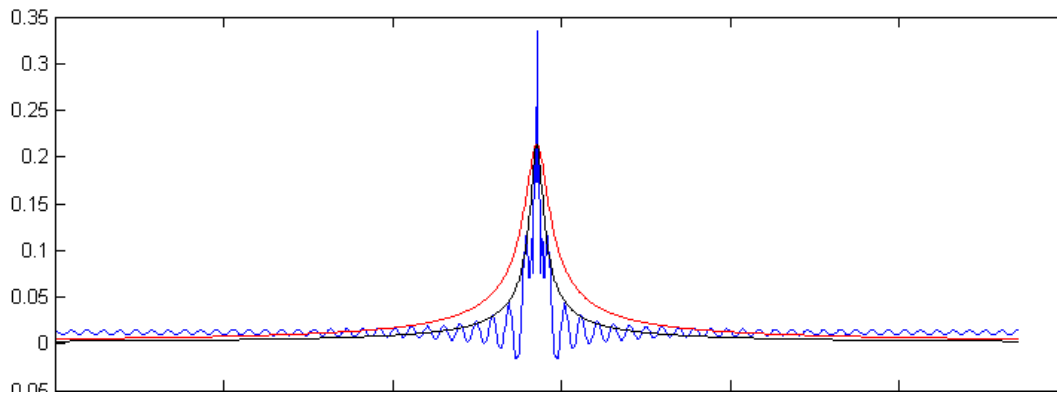
$$R^*(w) = G(w)H^*(w)$$

$G(w)$

$R(w)$

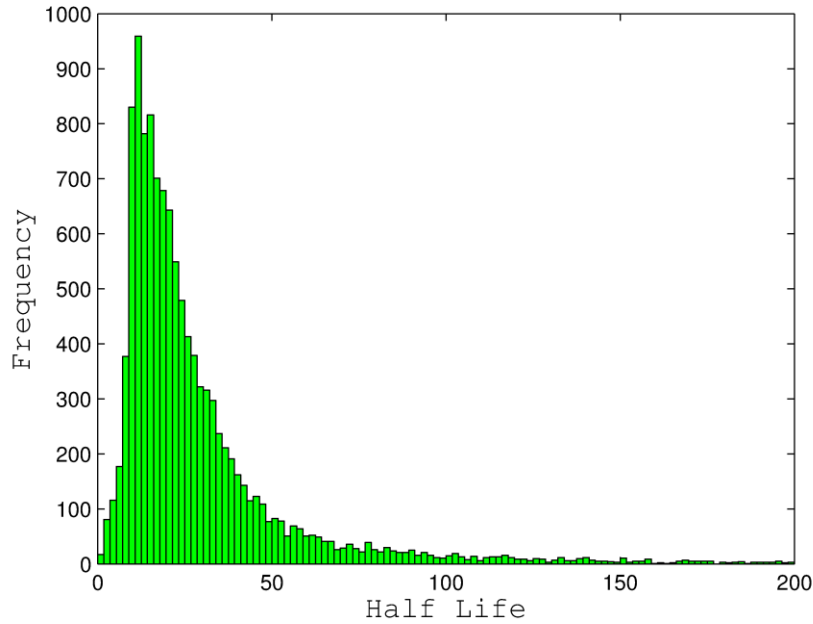
$$H(w) = R(w)/G(w)$$

$H^*(w)$  or  $H^*(w)$

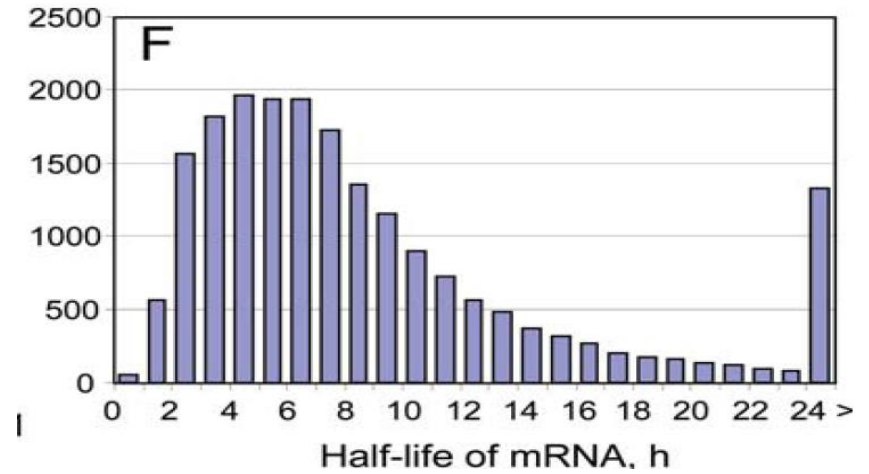


Transfer function in  
Fourier Domain

# Predicted mRNA half-lives

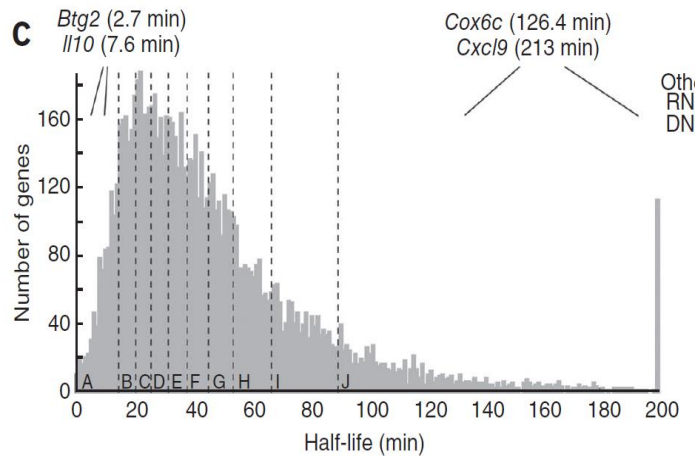


## Using ActD



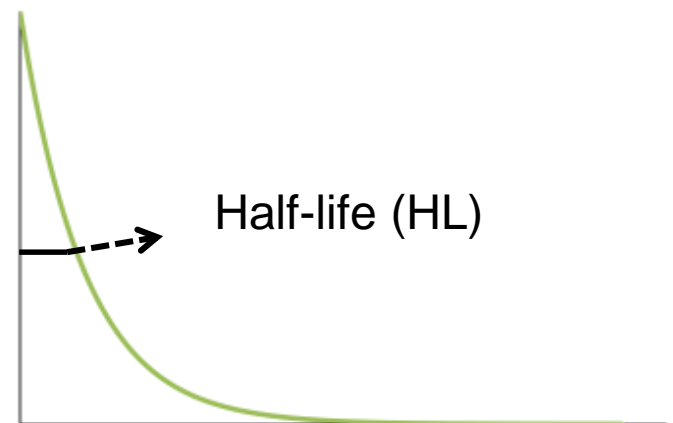
Sharova et al. DNA Res. 2009

## Using 4-thiouridine (4sU)



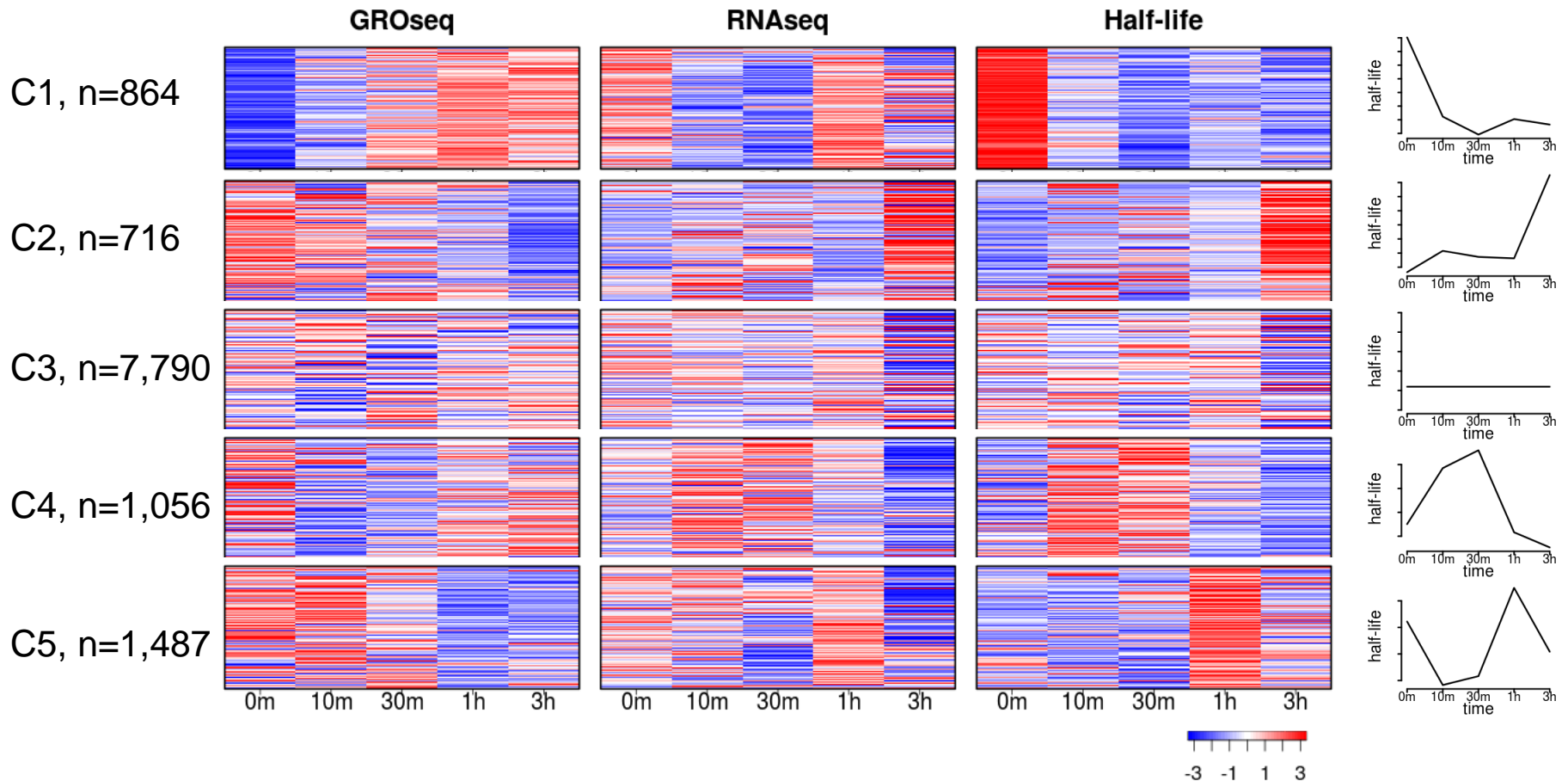
Rabani et al. Nat. Biotech. 2011

When  $G(t)=0$

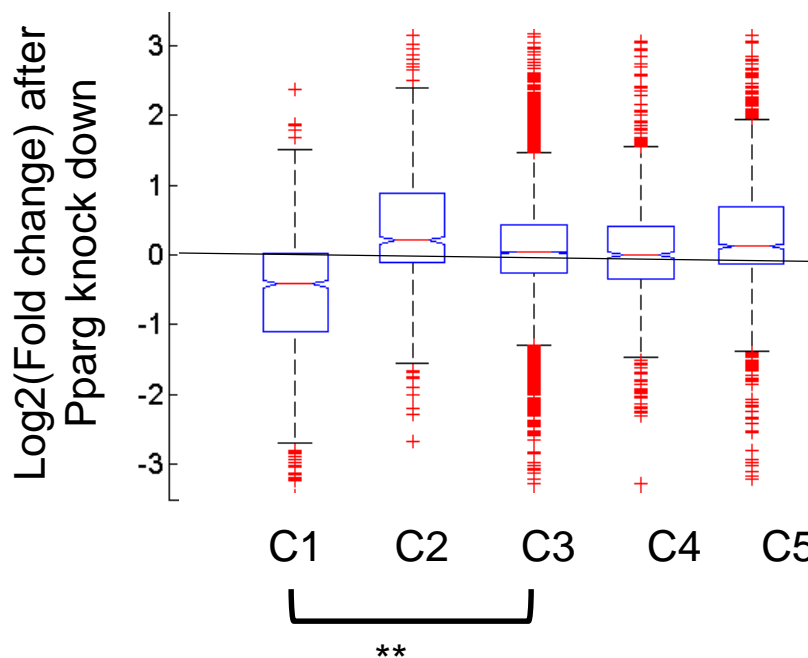
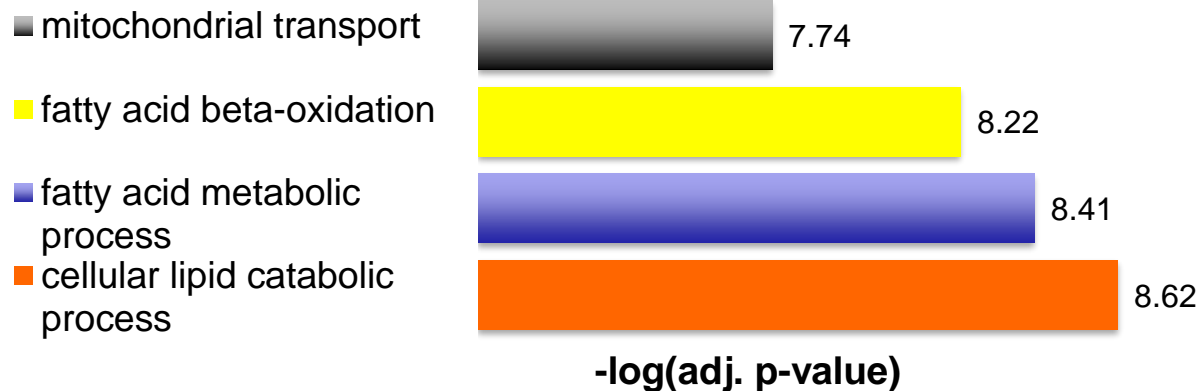
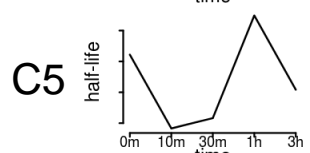
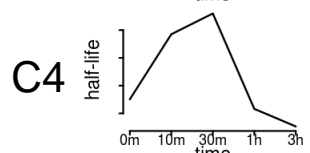
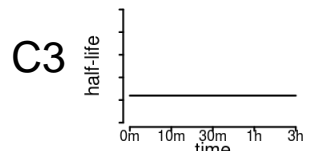
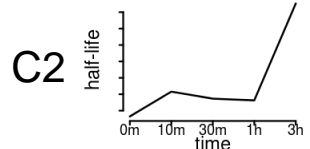
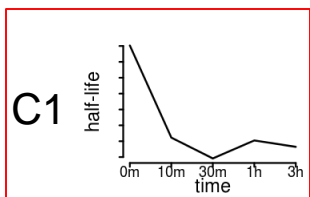


# Modeling variable time constant

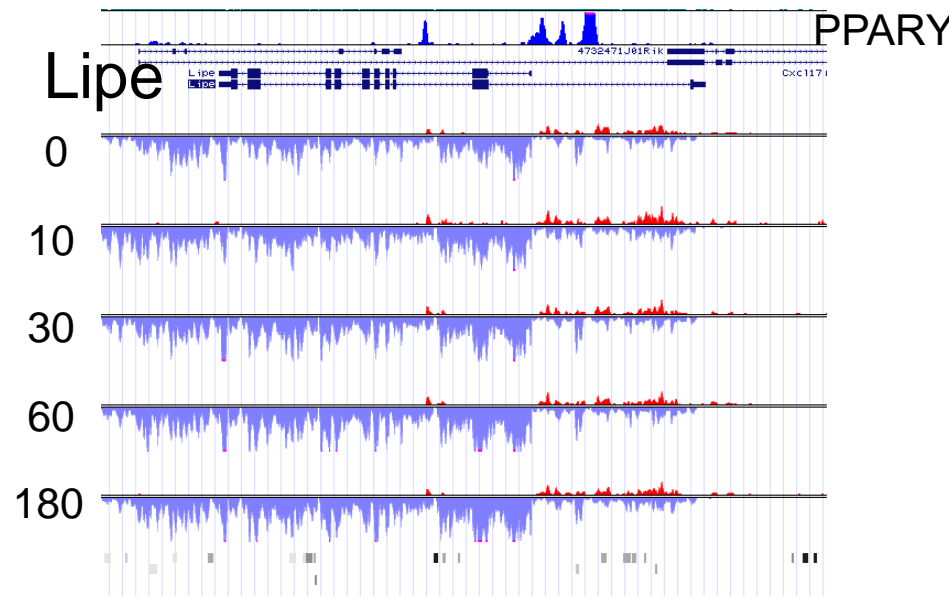
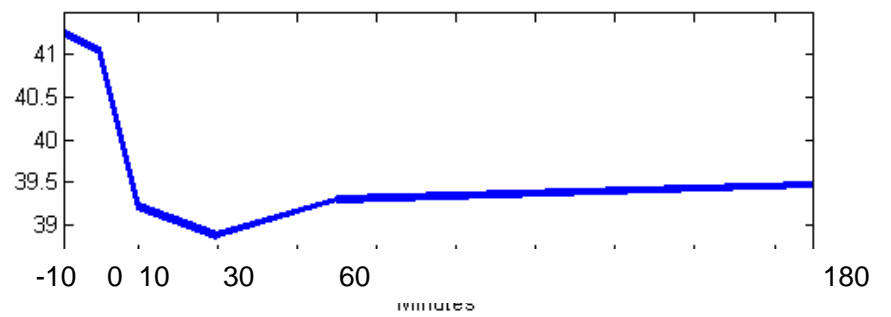
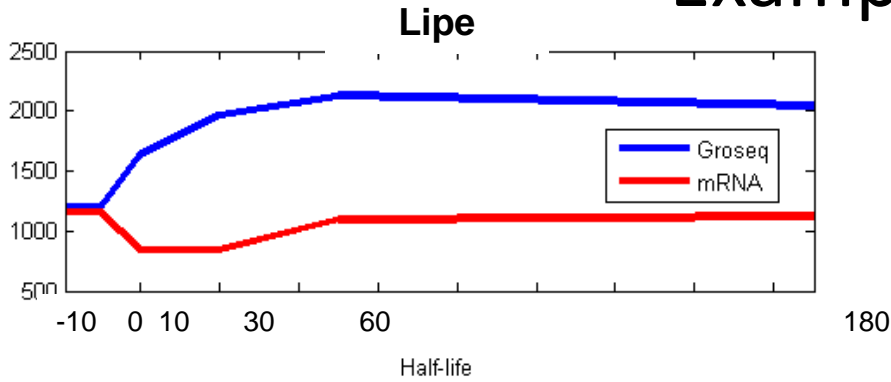
$$\frac{dR(t)}{dt} + \frac{R(t)}{T} = KG(t) \Rightarrow \frac{dR(t)}{dt} + \frac{R(t)}{T(t)} = K(t)G(t)$$



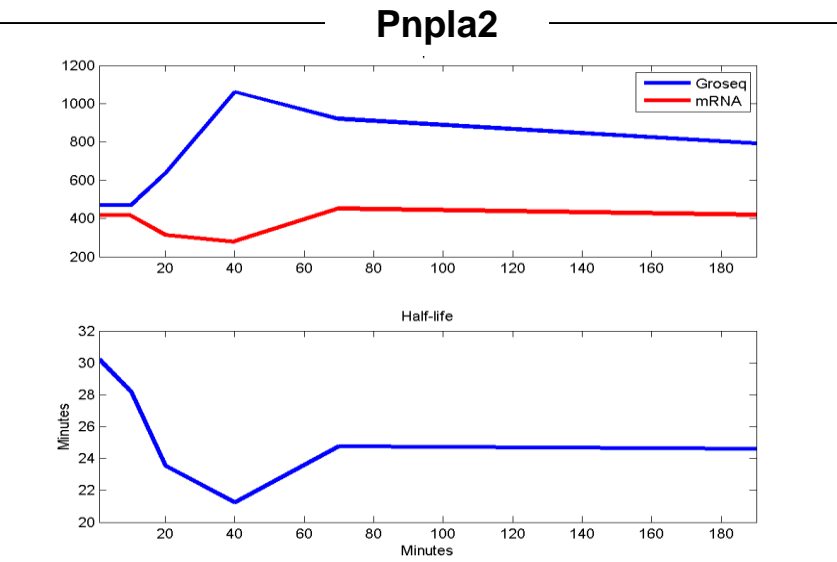
# Genes with reduced half lives are associated with metabolic process & many of them are targets of PPAR $\gamma$



# Examples: Lipe



GROseq levels were increased  
 RNAseq levels were remained  
 log2FC after KD: -3.22

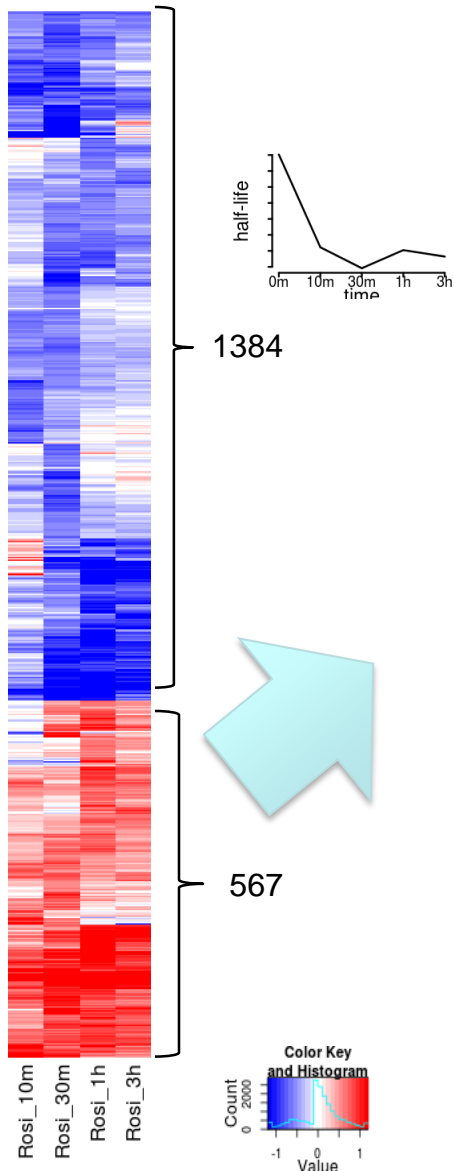


Lipe: hormone-sensitive lipase  
 Pnpla2: adipose triglyceride lipase  
 : related with lipolytic function

# Majority of genes induced early by rosi are belong the C1

Among the 567 genes, 320 are belong to C1

Log2-FoldChange  
in Gene Body



320 genes with  
decreasing stability

GO Biological Process

- small molecule catabolic process (3e-14)
- carboxylic acid catabolic process (2e-14)
- fatty acid catabolic process (3e-12)

Molecular function

- coenzyme binding (9.6e-11)
- NAD binding (0.00017), fatty-acyl-coA

KEGG

- fatty acid degradation (4e-8)
- fatty acid metabolism (1e-7)

247 genes without  
decreased stability

GO Biological Process

- positive regulation of glucose transport (0.007)
- regulation of glucose import (0.02)
- fatty acid biosynthetic process (0.09)

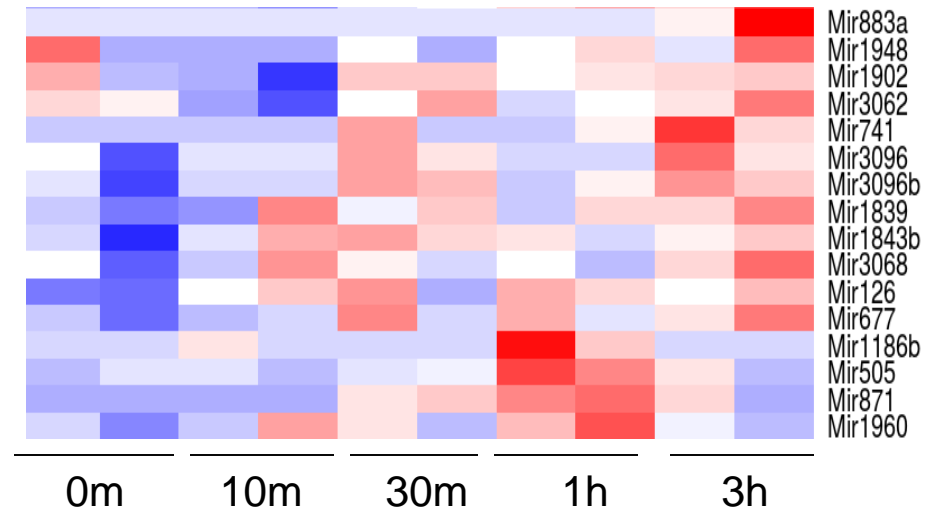
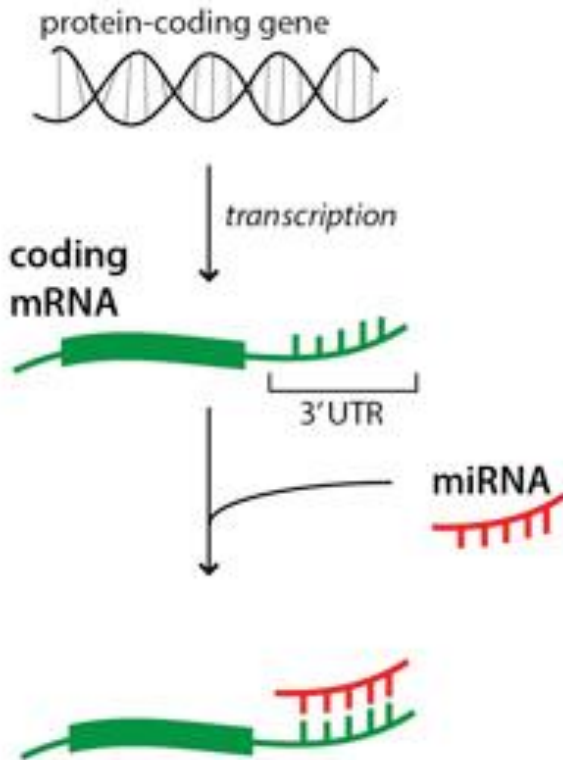
Molecular function

- protein kinase A catalytic subunit binding (0.2)

KEGG

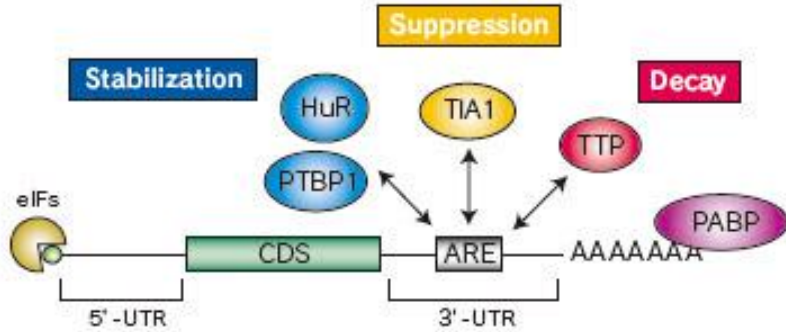
- PPAR signaling pathway (0.004)
- insulin signaling pathway (0.02)

# Degradation model I

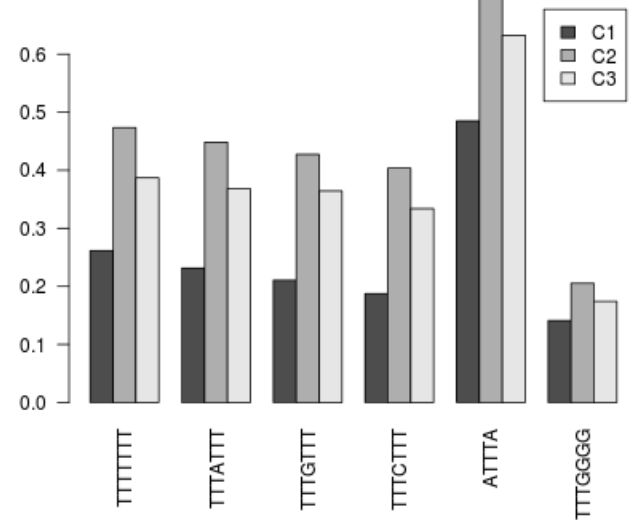


miRNA mediated degradation

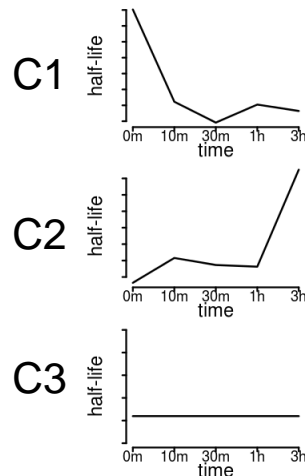
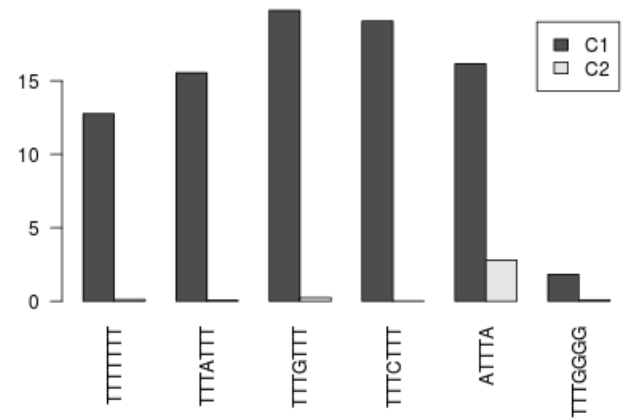
# C1 is depleted with AU-rich elements (AREs) compared with C2 and C3



**Ratio with motif**  
(Genes with ARE / total number of genes)



**Fisher's exact test**  
-log<sub>10</sub>(p-value)



Known HuR motif

negative control

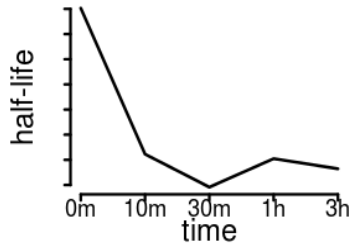


# Hypothesize: Genes with abrupt induction will lose its stability. But HuR stabilizes its target genes.

HuR motif

siHuR

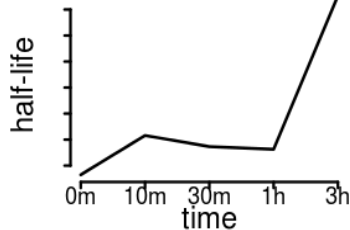
C1



no change

No change in expression

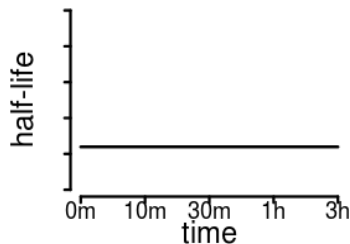
C2



Will reduce stability

No or less increase of expression

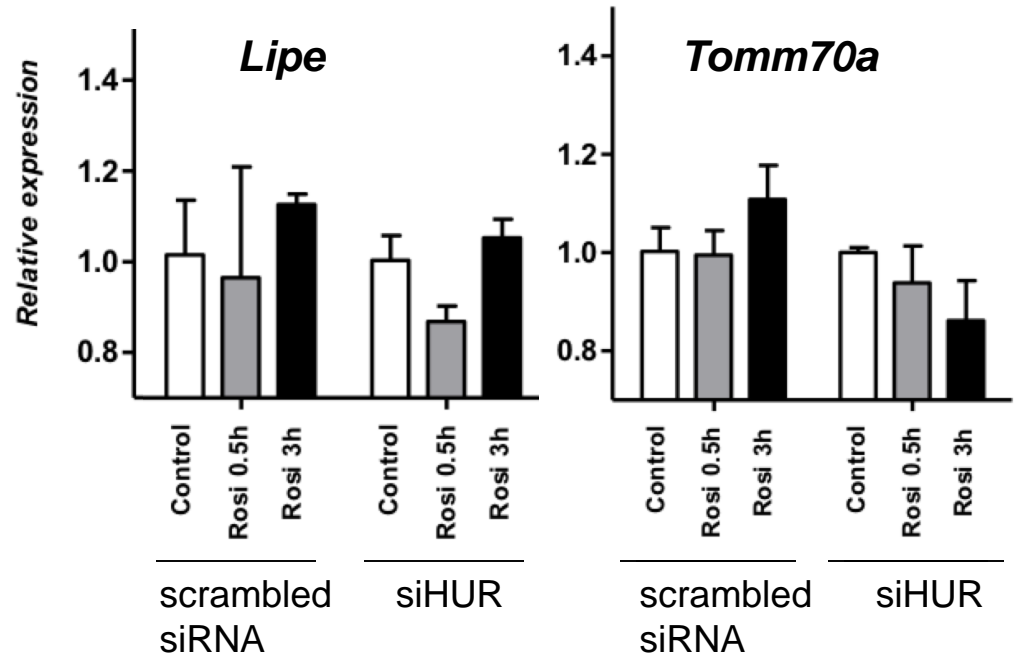
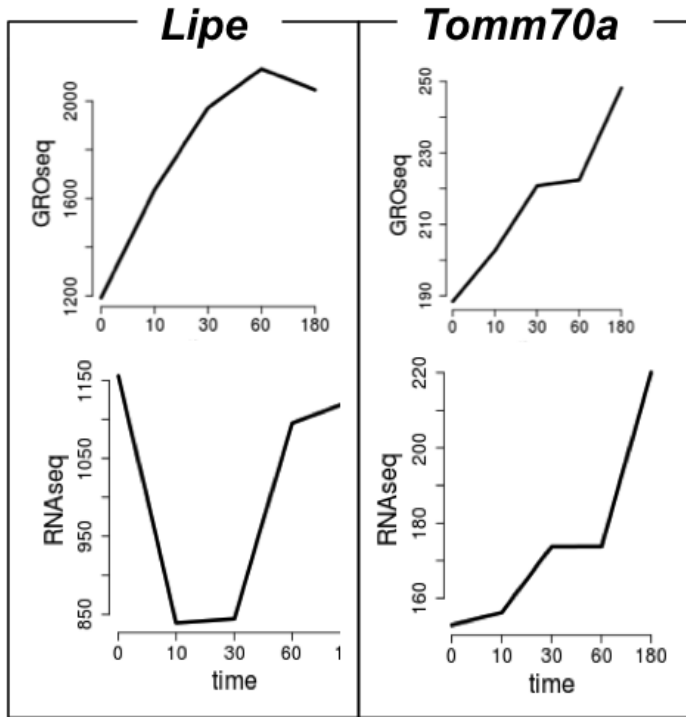
C3



Will reduce stability

No or less increase of expression

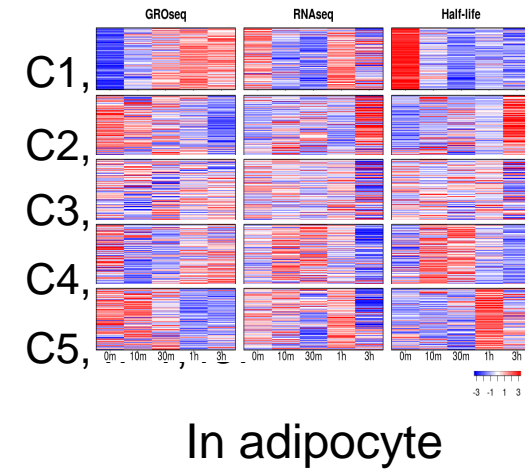
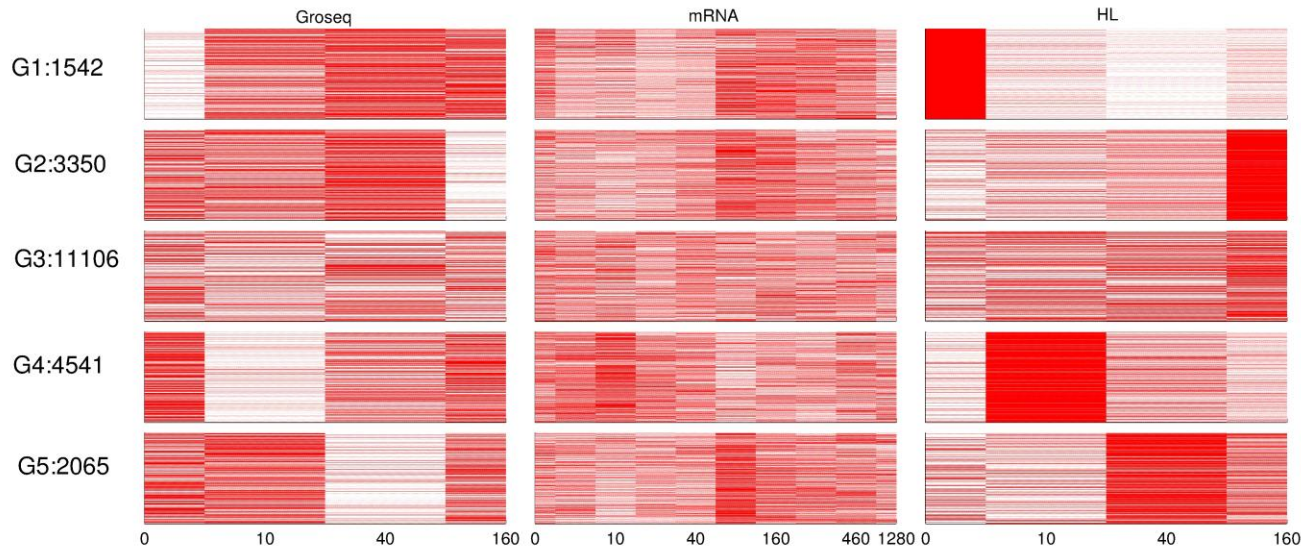
# siHuR in the mature adipocytes block the increase of expression only in Tomm70a



C1: No increase in expression

Non-C1: increase in expression

# E2 treatment in breast cancer cell lines (MCF7)



GROseq: from Lee Kraus Lab. (Hah et al. 2011 Cell)

RNAseq : from Rattray lab (Honkela et al. 2015, PNAS)

# Why?

RNA homeostasis

Similar to protein homeostasis

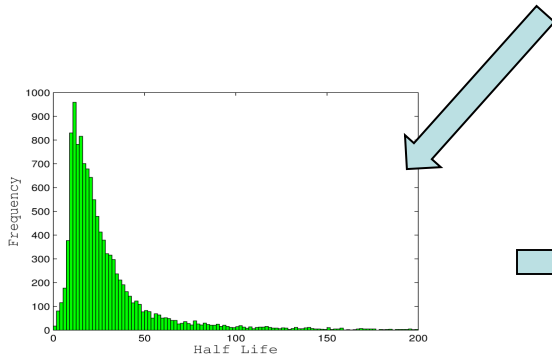
=> The changes in expression levels do not guarantee the changes in the protein levels.

# Summary

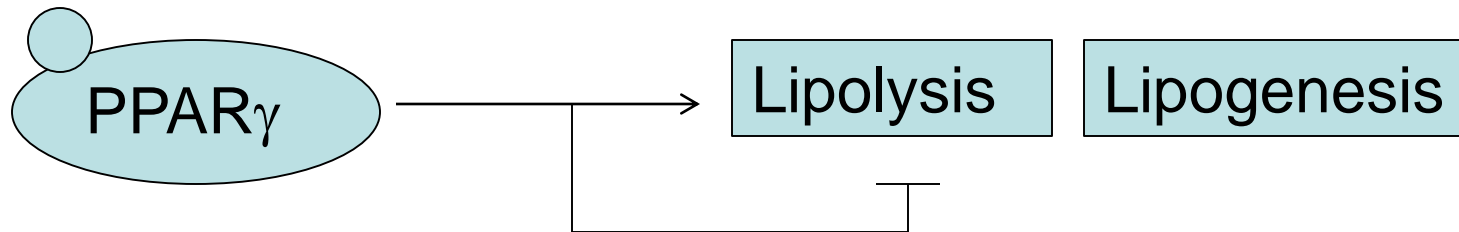
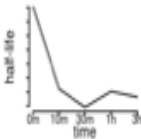
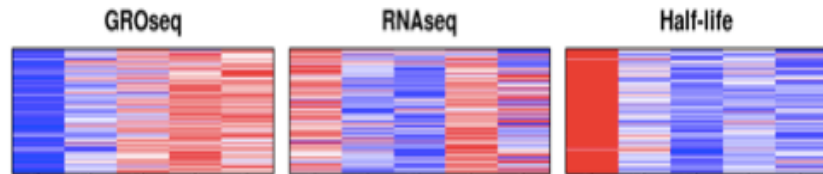
Transcription Level  
(Groseq;  $G(t)$ )

Transfer  
function (H)

Expression Level  
(RNAseq;  $R(t)$ )

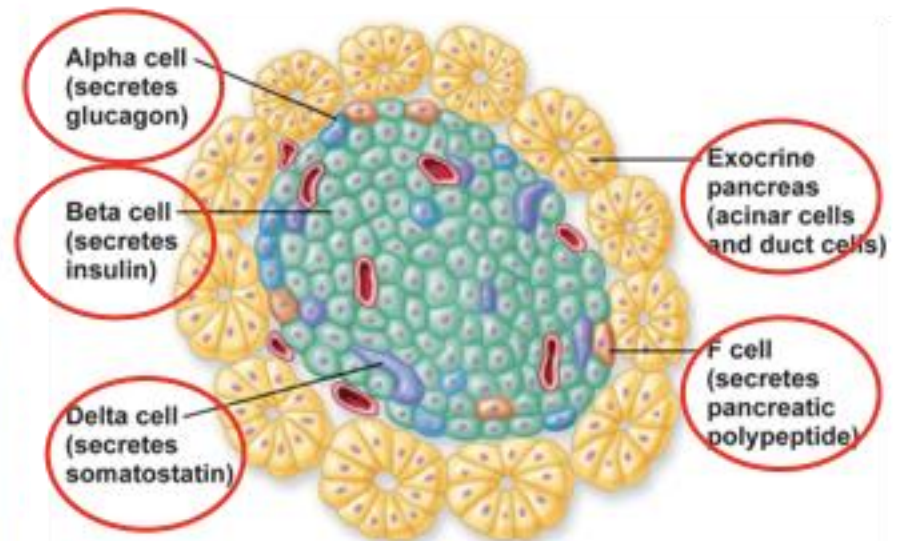
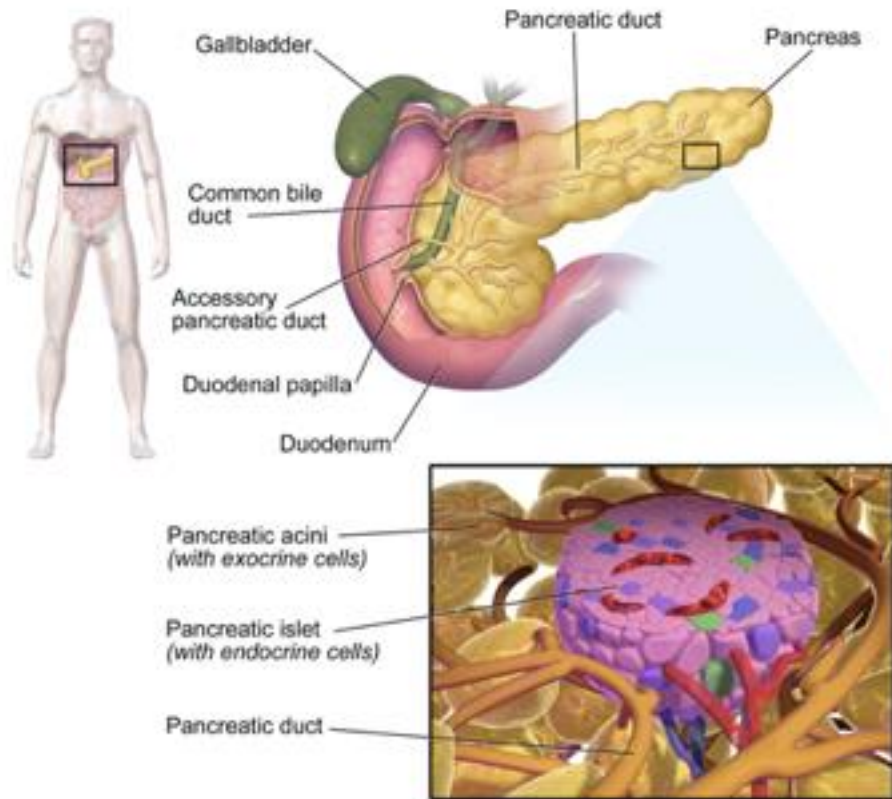


C1, n=864



- Not all PPAR $\gamma$  binding sites are responsive to its agonist

# Pancreatic tissue



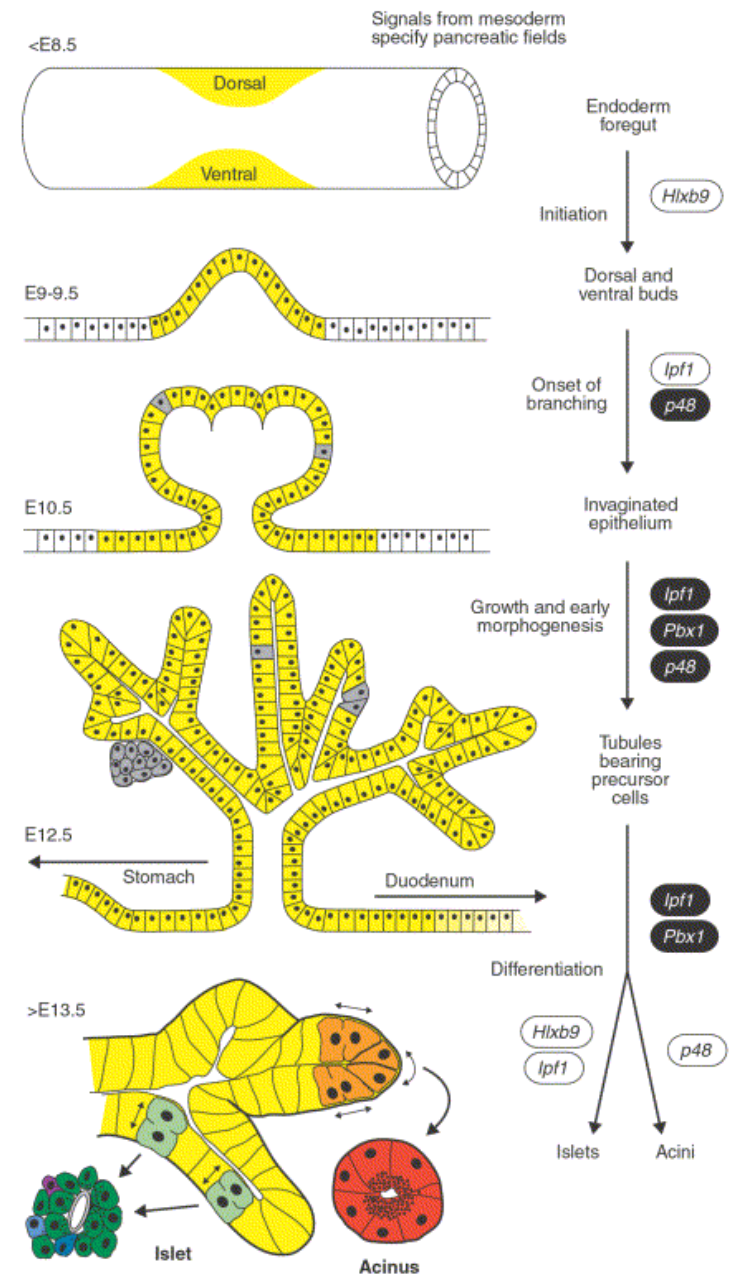
<http://quasargroupconsulting.com/anatomy/pancreas.php>

**Pancreatic Tissue**

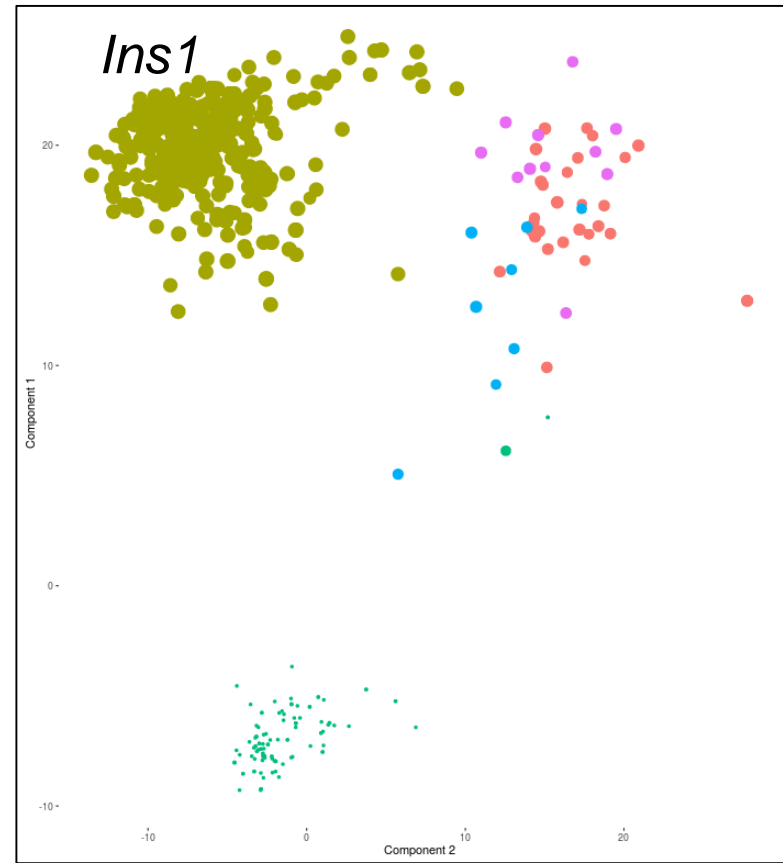
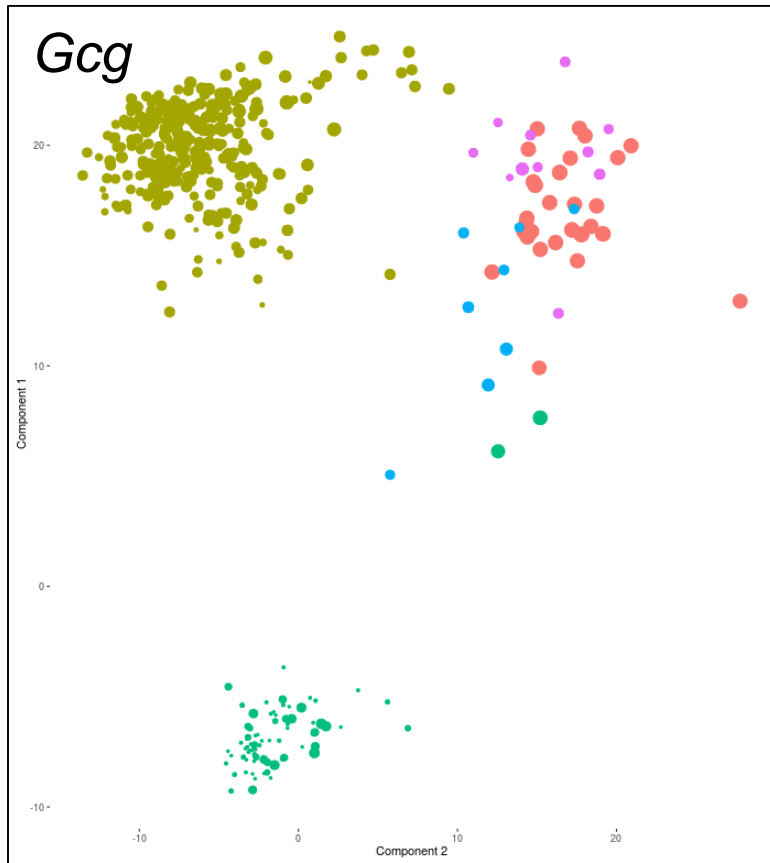
# Single cell RNAseq for mouse pancreatic epithelial cells at e13.5

Total 77 single cells.

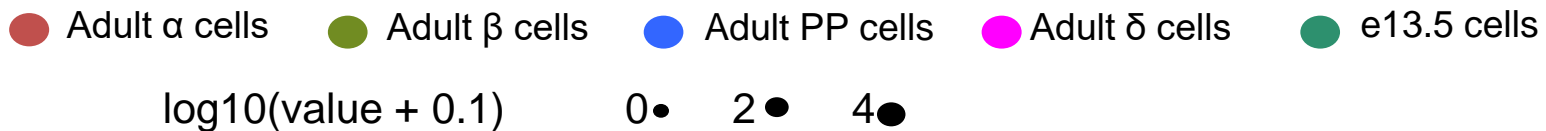
At e13.5, the pancreatic progenitors express endocrine progenitors of each lineage



# Clustering scRNAseq from pancreas



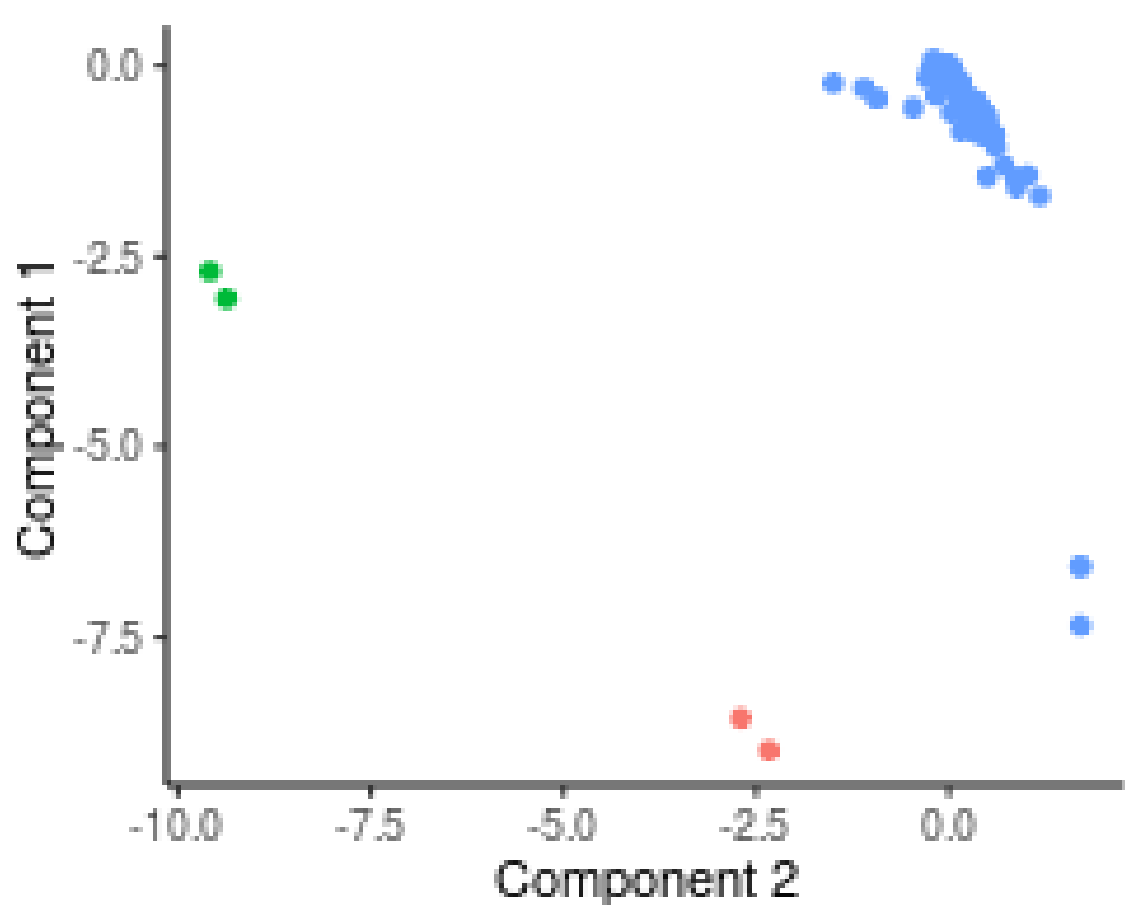
Adult  $\alpha$  cells and  $\beta$  cells are from Xin et al. (2016) PNAS





# Clustering with RNAseq data identifies cells committed to $\alpha$ cells

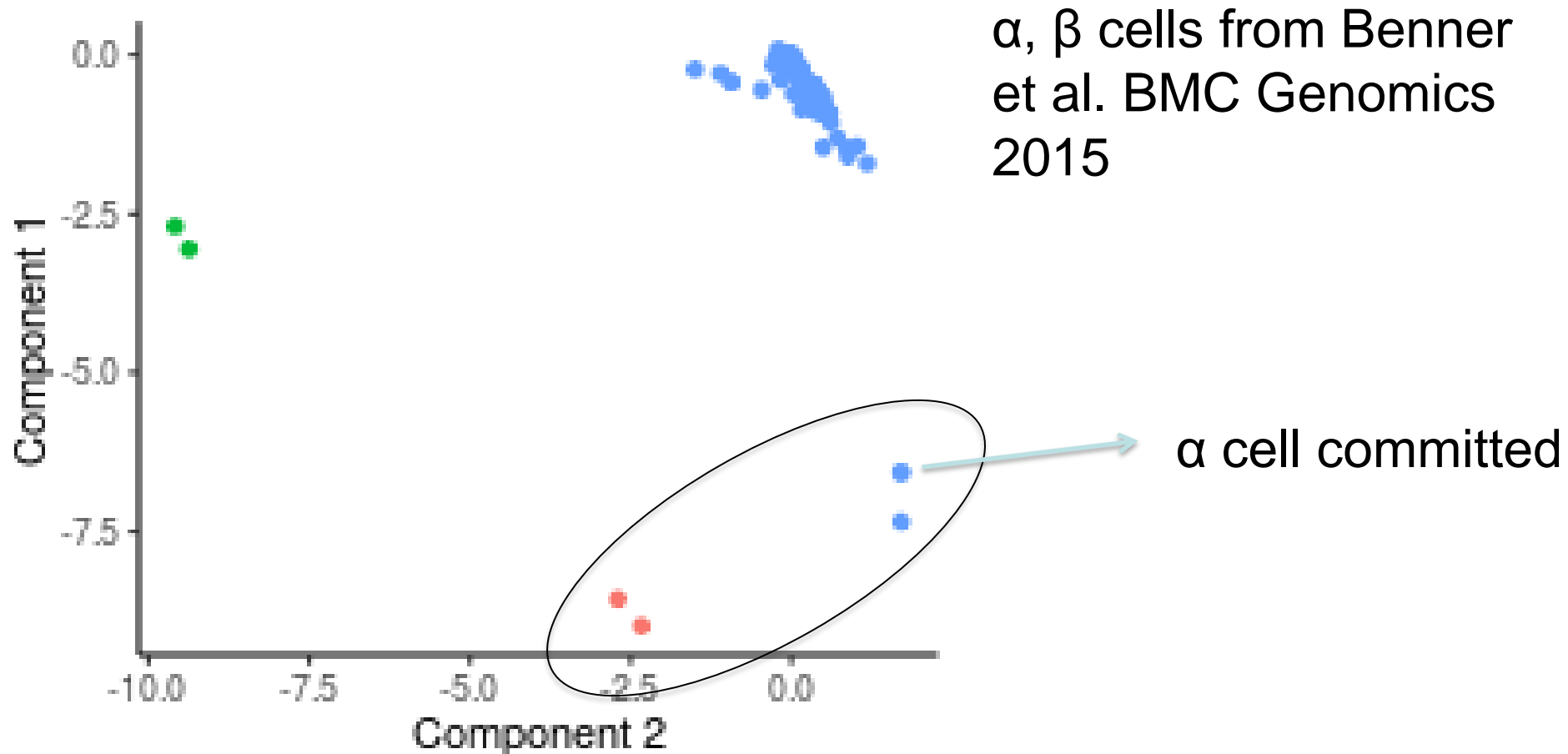
•  $\alpha$     •  $\beta$     • Single cells



$\alpha$ ,  $\beta$  cells from Benner et al. BMC Genomics 2015

# Clustering with RNAseq data identifies cells committed to $\alpha$ cells

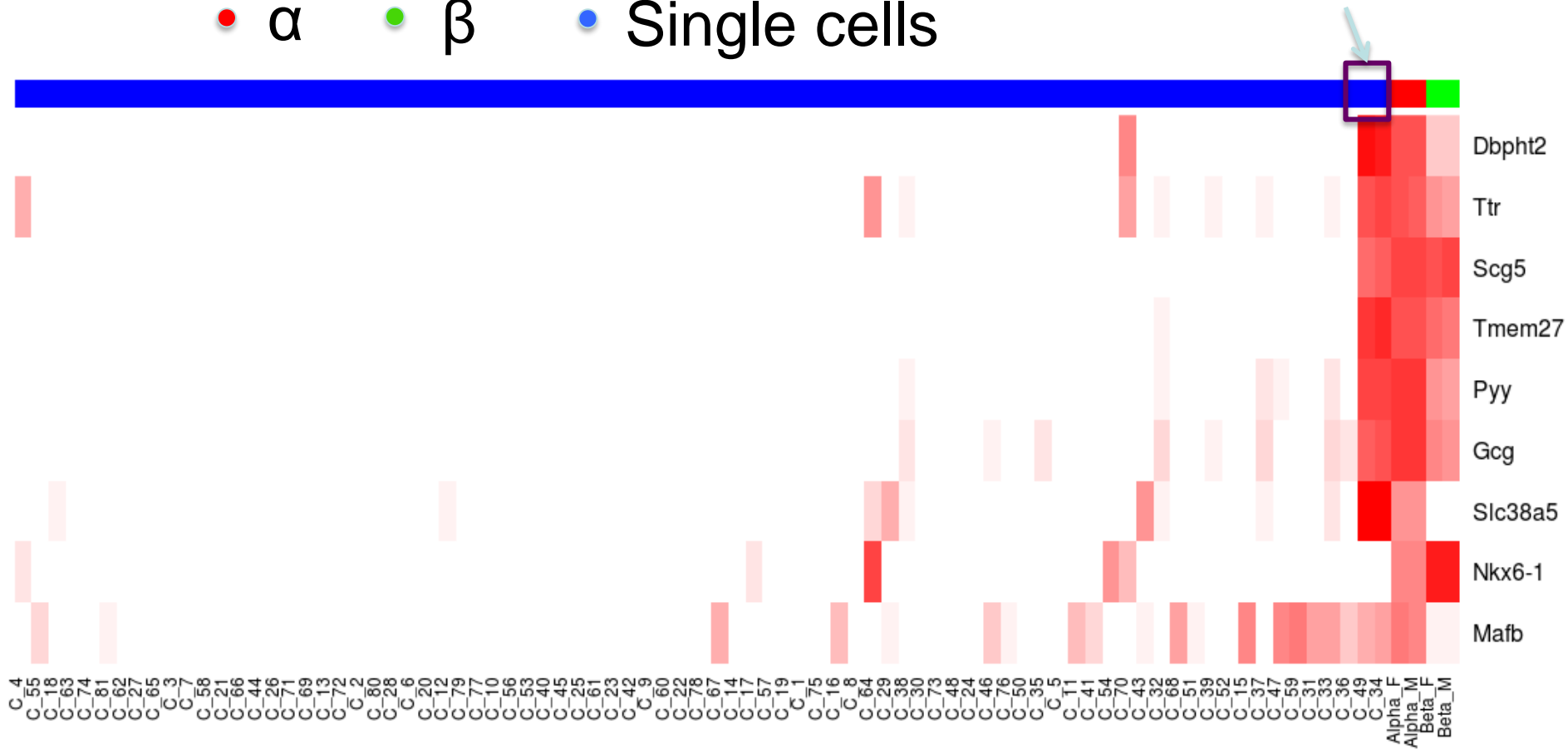
•  $\alpha$    •  $\beta$    • Single cells



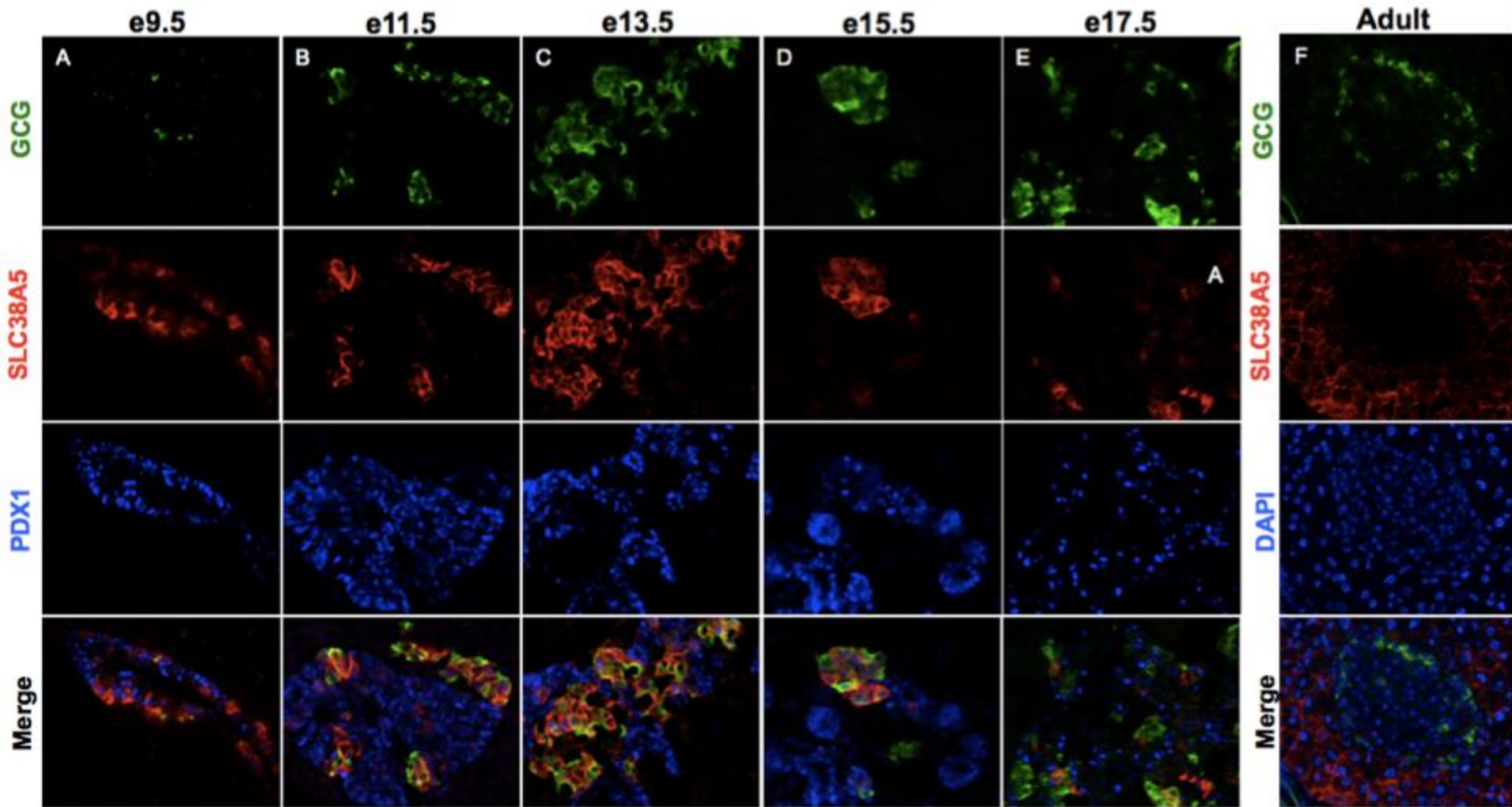
Slc38a5 correlates with Gcg and is highly observed in the  $\alpha$  cell committed cells

●  $\alpha$  ●  $\beta$  ● Single cells

$\alpha$  cell committed cell

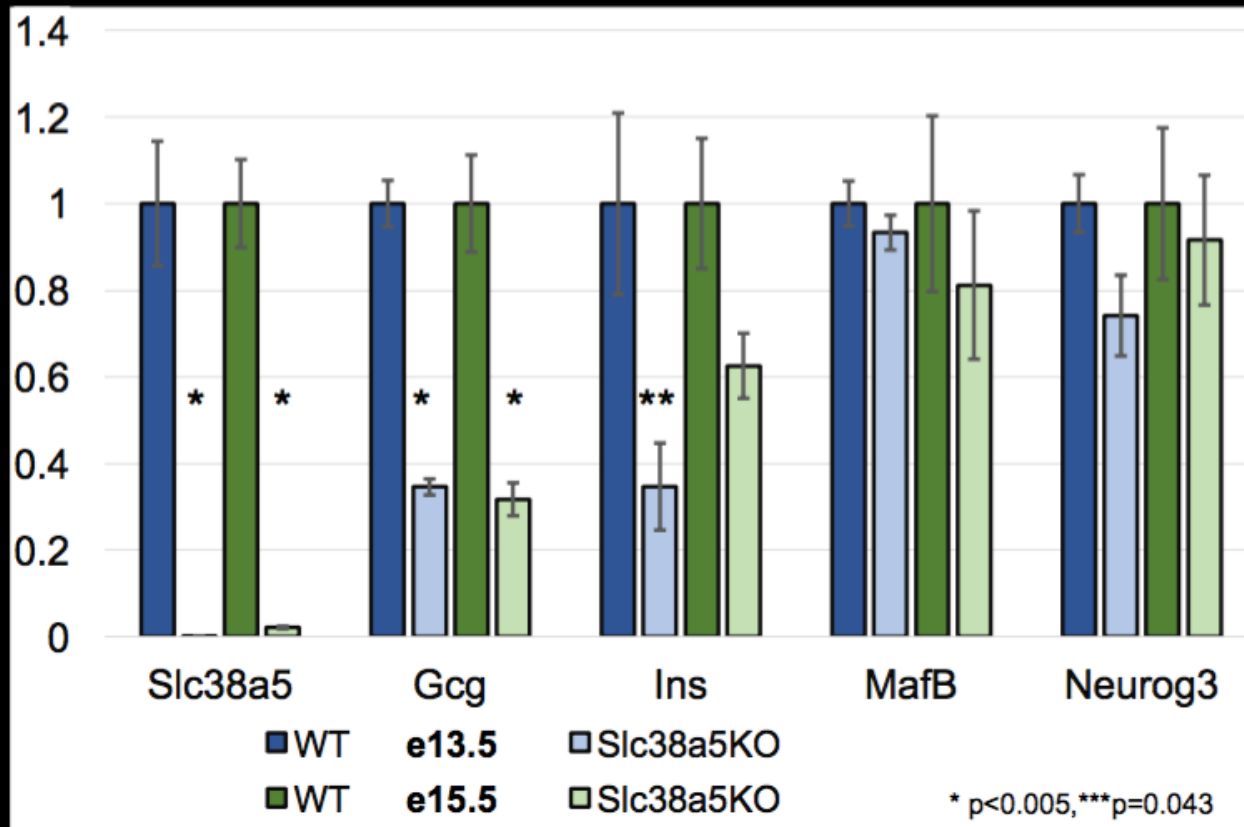


SLC38A5 (Snat5) is uniquely and highly expressed in the developing pancreas until late gestation



Stanescu et al.

# Loss of SLC38A5 impairs Gcg expression throughout mid-gestation



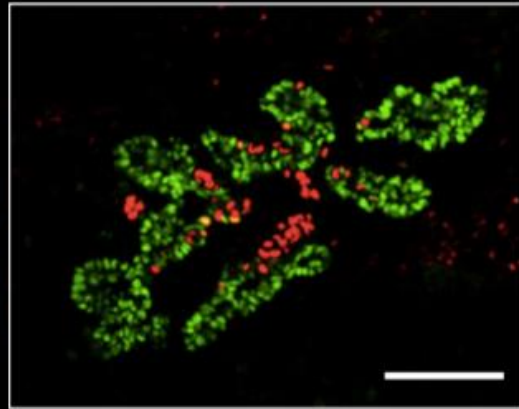


# Loss of SLC38A5 leads to decreased numbers of MafB progenitors

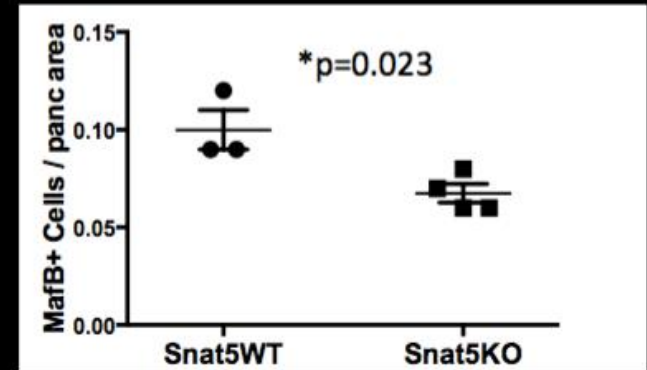
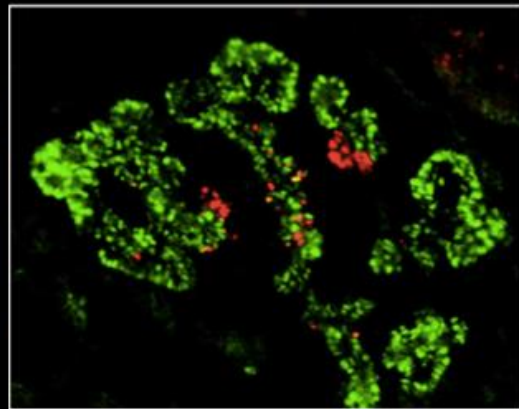
Pdx1 MafB

e13.5

*Slc38a5*WT



*Slc38a5*KO



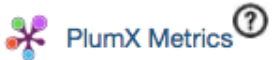
ARTICLE

# Amino Acid Transporter Slc38a5 Controls Glucagon Receptor Inhibition-Induced Pancreatic $\alpha$ Cell Hyperplasia in Mice

Jinrang Kim, Haruka Okamoto, ZhiJiang Huang, Guillermo Anguiano, Shihwei Chen, Qing Liu, Katie Cavino, Yurong Xin, Erqian Na, Rachid Hamid, Joseph Lee, Brian Zambrowicz, Roger Unger, Andrew J. Murphy, Yan Xu, George D. Yancopoulos, Wen-hong Li<sup>4</sup>, Jesper Gromada<sup>4,5</sup>

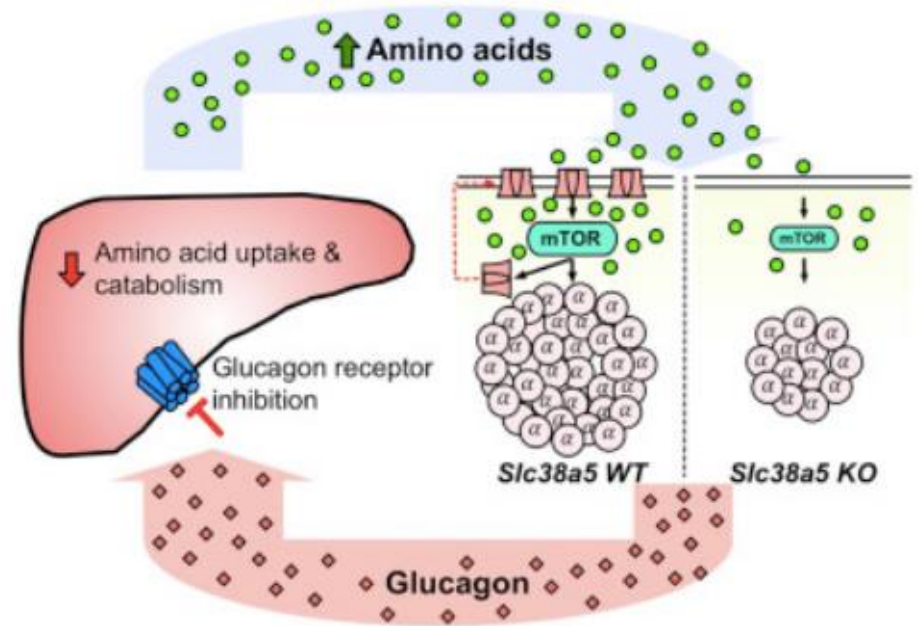
<sup>4</sup> Senior author

<sup>5</sup> Lead Contact



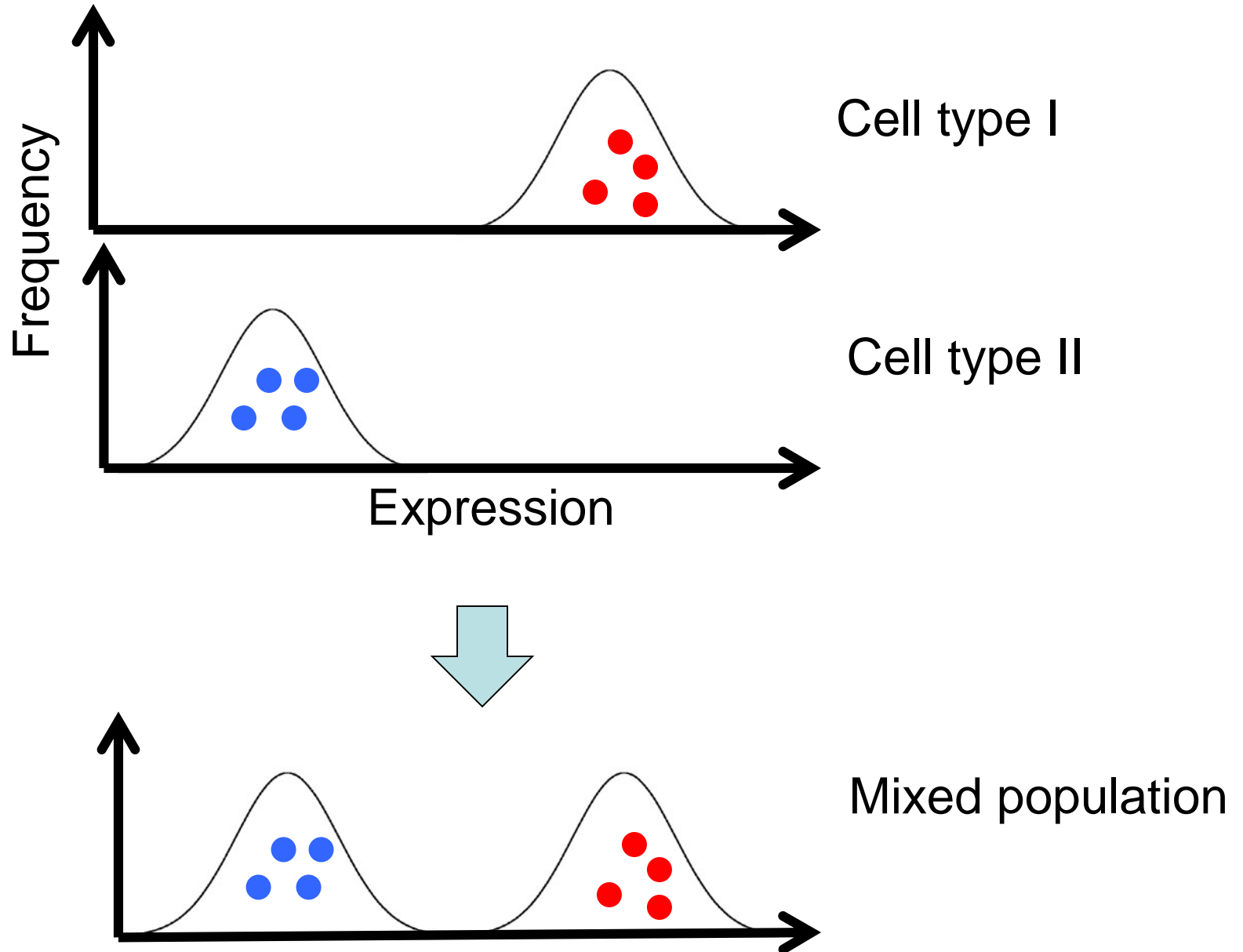
DOI: <https://doi.org/10.1016/j.cmet.2017.05.006> | CrossMark

Article Info

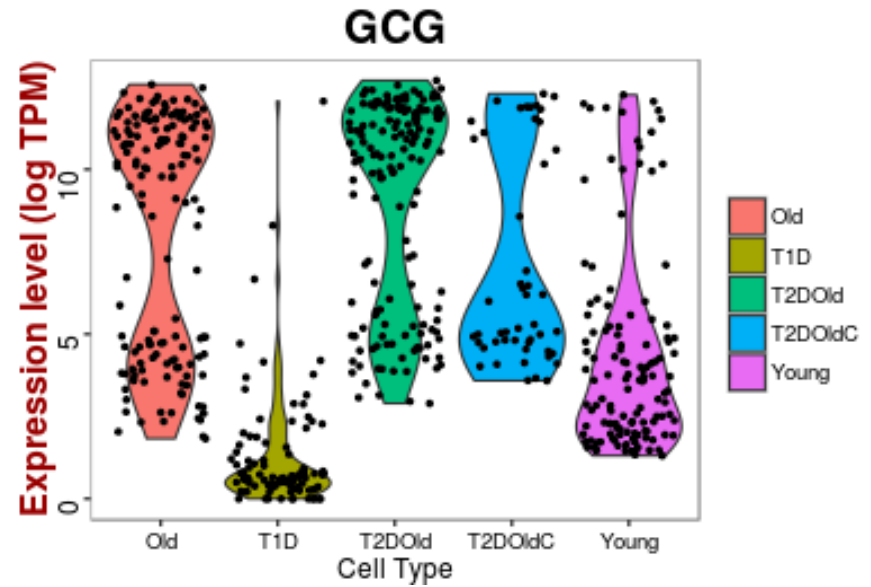
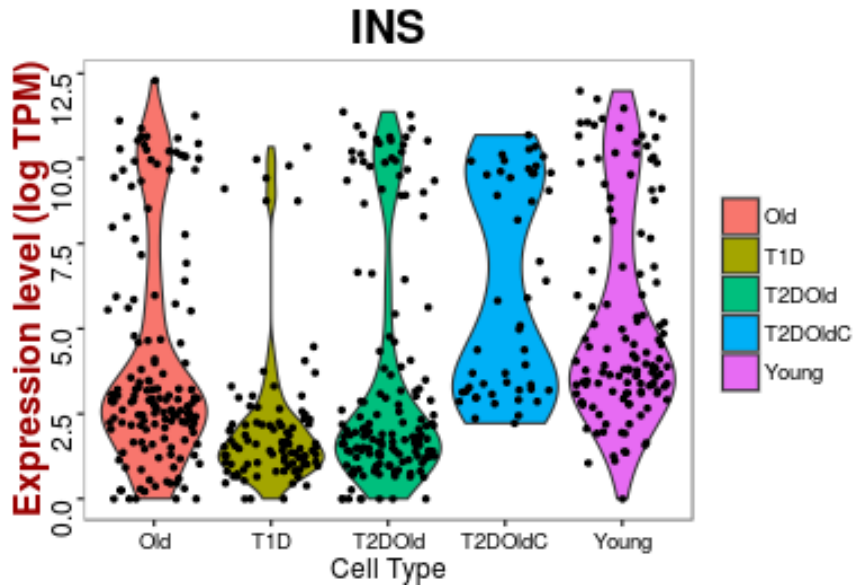




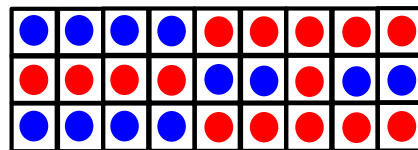
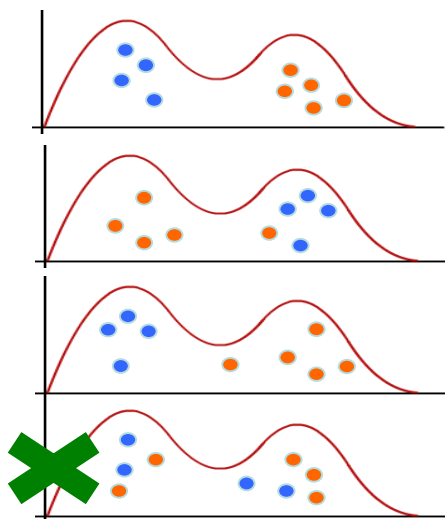
# Bimodal distribution is observed when 2 cell types are mixed in scRNAseq



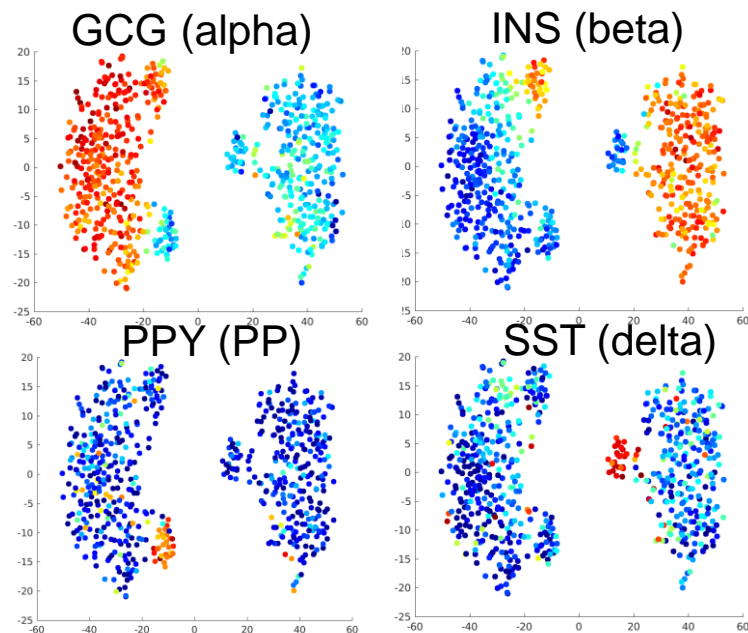
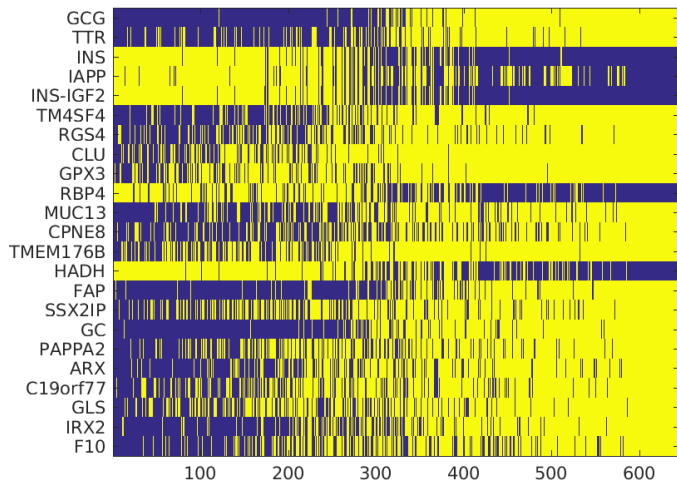
# scRNAseq data from pancreatic islet show bimodal patterns for key genes



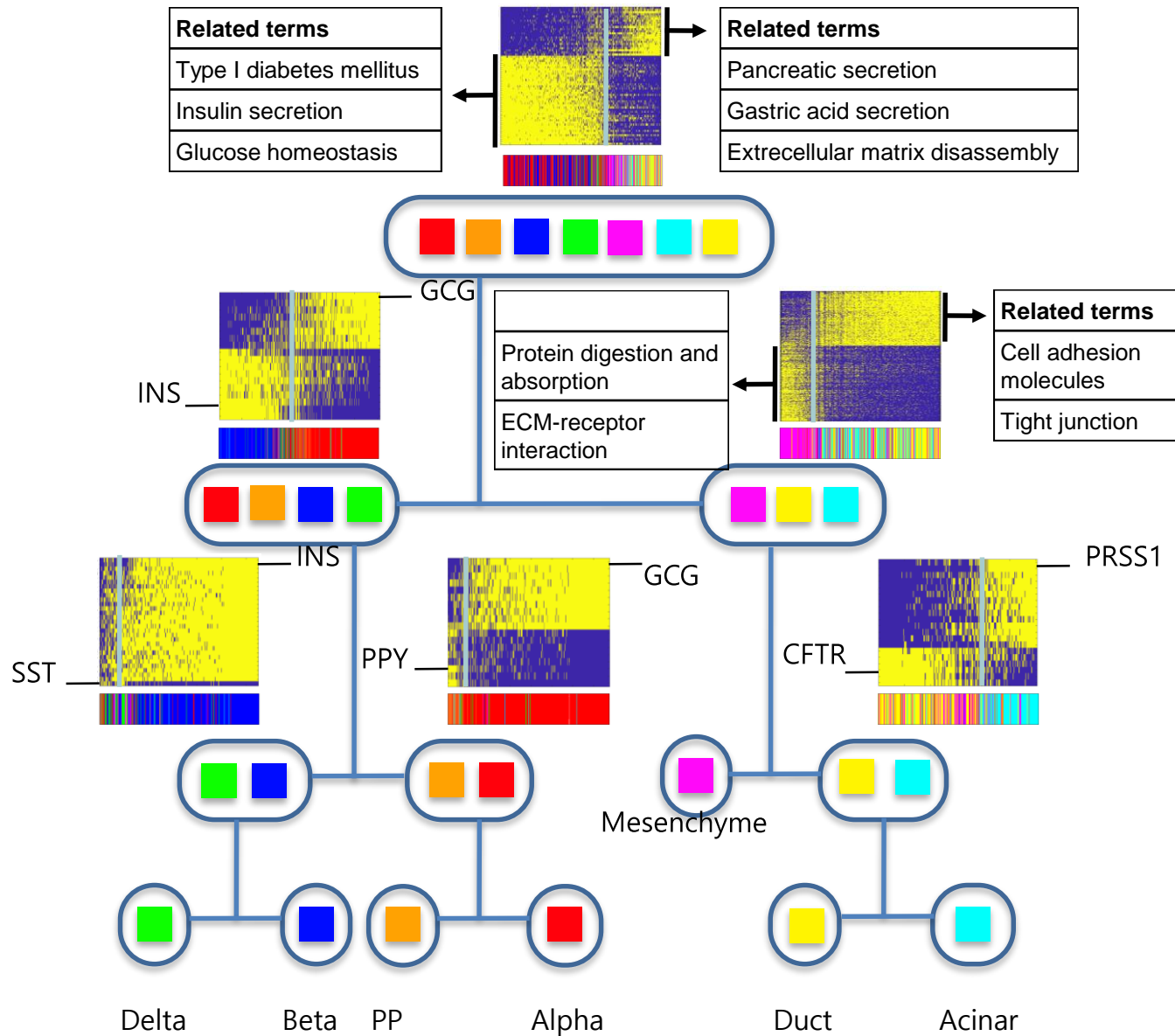
# A new clustering algorithm using bimodal distribution



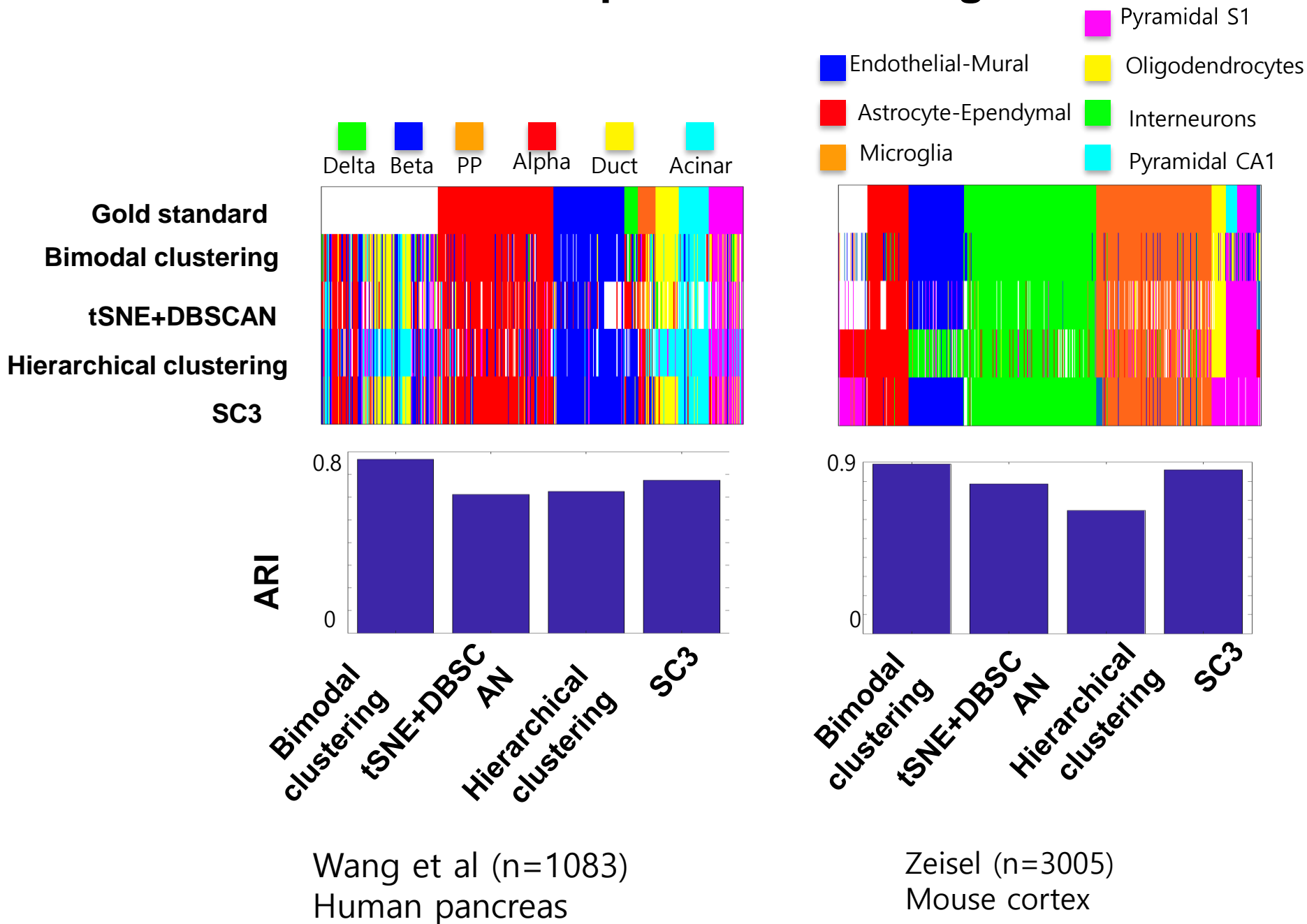
Coding with 0 or 1



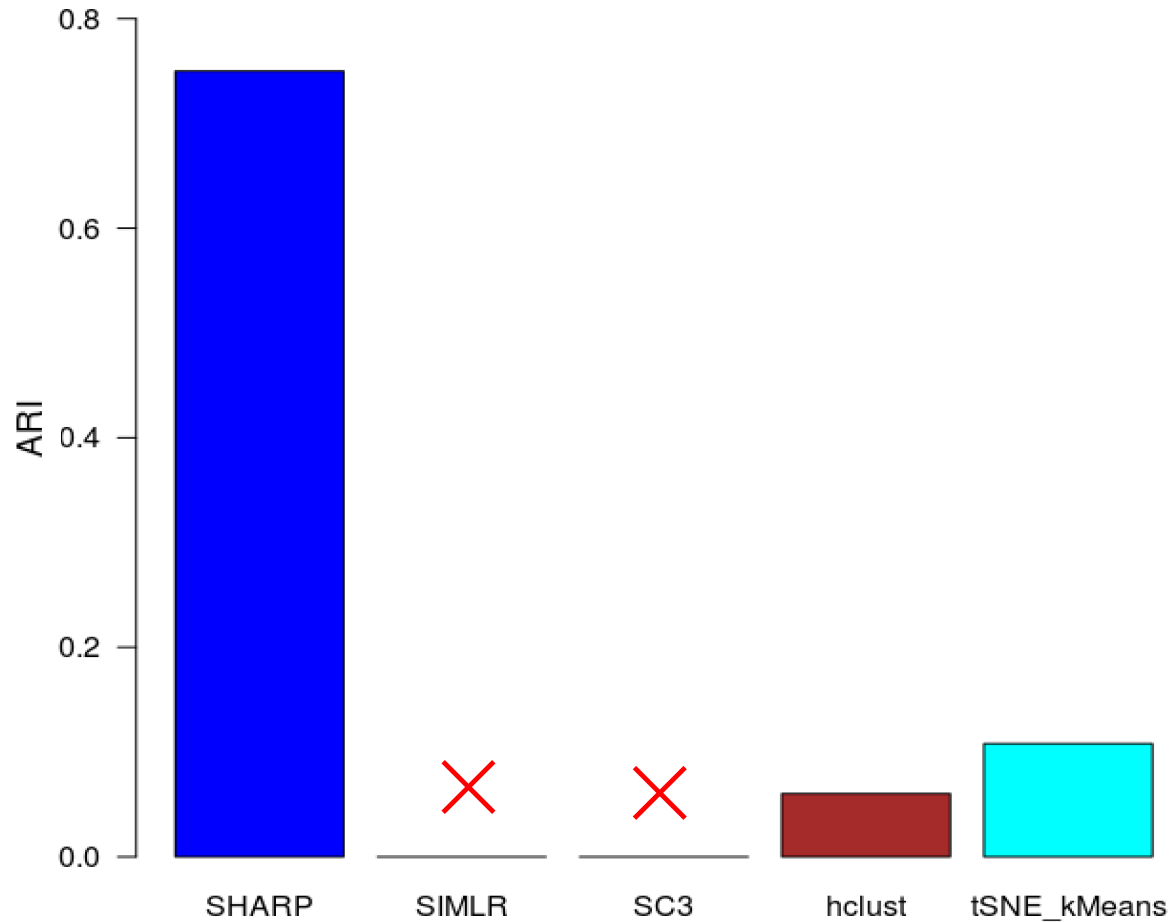
# A top-down clustering for human pancreatic islet data



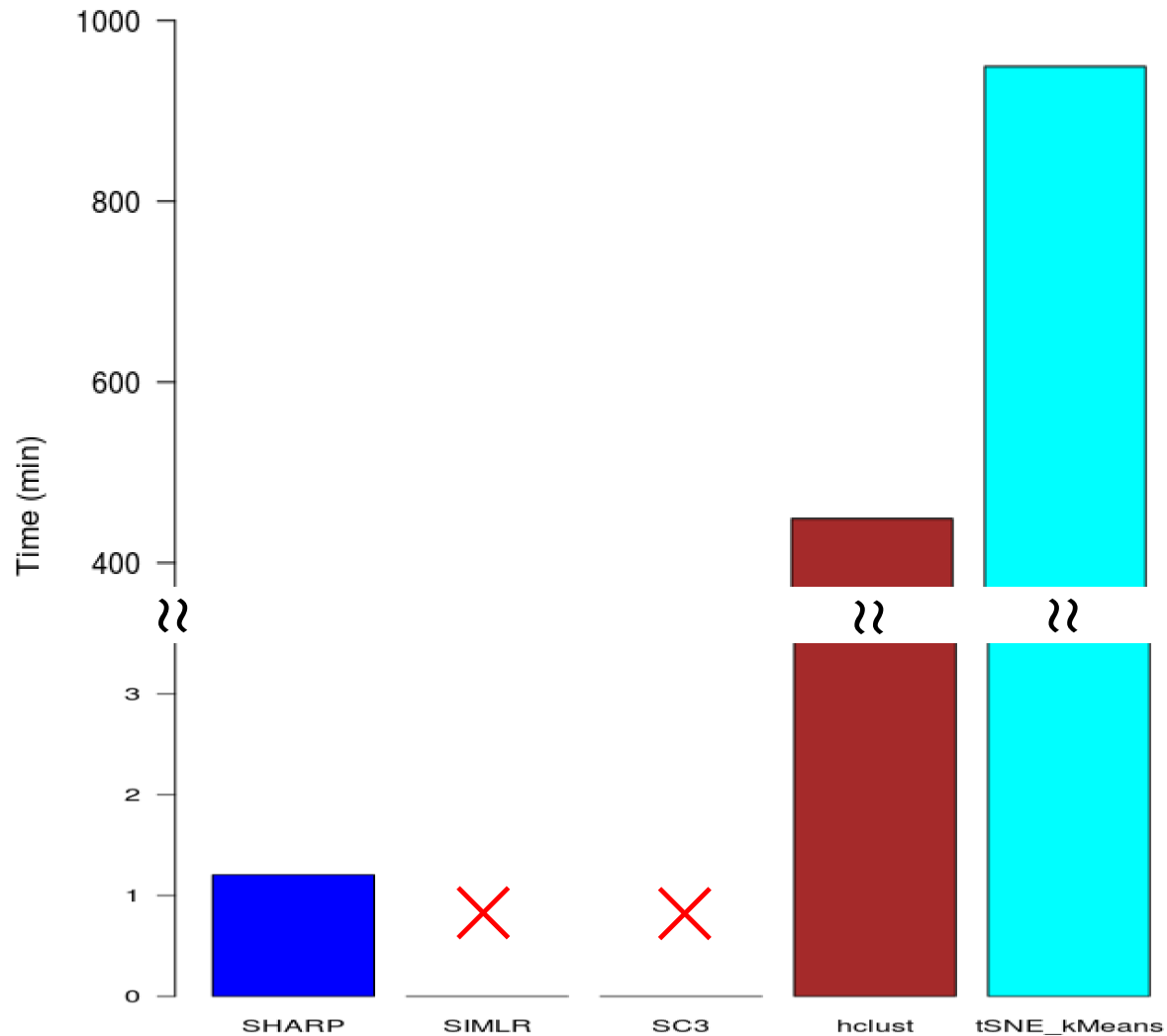
# Evaluation of the top-down clustering



# SHARP for Park (ARI)



# SHARP for Park (Time)



# Thanks to

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Sajid Marhon

Shibiao Wan

**Nha Nguyen**

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**Doris Stoffers**

**Diana Stanescu (CHOP)**

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**Montserrat Anguera**

- X chromosome inactivation

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**Mitchell Lazar**

David Steger

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**National University)**

Hee-jai Shin



**R01 DK106027**



**ITMAT (UL1 TR001878)**



**P30 AI45008-18**

