Differential dynamics of the mammalian mRNA and protein expression response to misfolding stress

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Abstract

问题	The relative importance of regulation at the mRNA versus protein level
解决方法	mapped proteomic and transcriptomic changes in mammalian cells responding to stress induced by dithiothreitol over 30 h
过程	1、estimated the kinetic parameters for the synthesis and degradation of RNA and proteins 2、 deconvoluted the response patterns into common and unique to each regulatory level
结果	1、equally important 2、 differed in their impact on molecule concentrations
具体结果	1、peaked between two and eight hours, 2、mRNA concentrations shifted in a transient, pulse-like pattern 3、protein concentrations switched only once and established a new steady state
进展	hypotheses on specific regulatory modes for some genes

CONTENTS

01

Background

技术发展：genome-wide mRNA and protein concentration data over multiple conditions or in a time course

课题进展：1、monitor the progression of mouse liver cells through the cell cycle (Robles et al, 2014) and the response of dendritic cells to lipopolysaccharide (LPS) treatment 2、RNA-level regulation appeared to be stronger than that of protein-level changes, fueling the debate on the relative importance of transcription, translation, and degradation.

PECA：protein expression control analysis

1、dissects mRNA- and protein-level regulation in time-resolved analyses

2、computes the ratio of synthesis and degradation rates

ER stress：The ER is the major protein-folding machinery and therefore highly sensitive to reagents that challenge protein folding, such as dithiothreitol (DTT). The ER stress response plays a crucial role in numerous human diseases, for example, hypoxia, ischemia reperfusion injury, heart disease,diabetes, and neurodegenerative diseases such as Alzheimer’s and Parkinson’s, in which prolonged protein misfolding is detrimental to the cell

02

Materials and Methods

Materials and Methods

01 Cell culture and experimental setup

02 Cell counting

03 DNA staining and flow cytometry

04 Immunocytochemistry

05 Transcriptomics measurements

06 Transcriptomics data processing and quality control

Materials and Methods

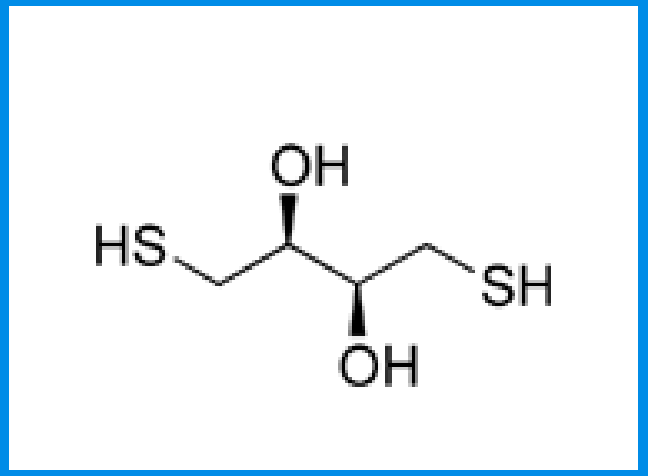
07 Proteomics experiments

08 LC-MS/MS analysis

09 Proteomics data processing and quality control

10 Proteomics data processing and quality control

11 Protein expression control analysis

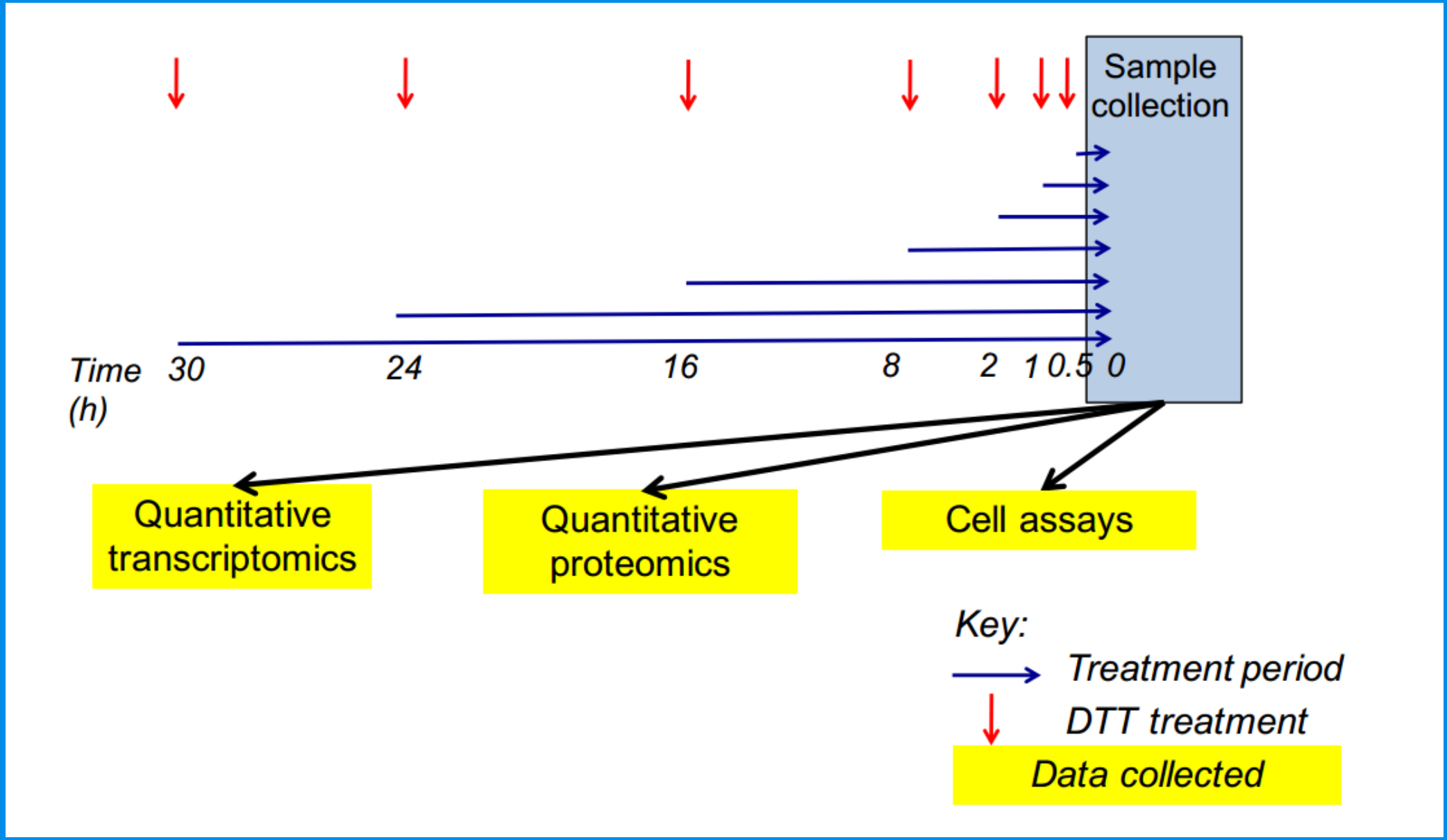


DTT二硫苏糖醇

1、DT T作为一种强还原剂 ,其 - SH基极为活泼 ,在室温下极易氧化重新生成 - S- S- 键

2、DT T 通过干扰发生于内质网的氧化 - 还原反应而影响二硫键形成及蛋白质折叠, 大量折叠障碍的蛋白质堆积于内质网引起内质网应激反应 (ER stress), 而 ER stress可激活细胞的自我挽救措施, 如通过减少蛋白质的生成或促进蛋白质的排出以缓解现状 , 当无力挽救时 ,细胞即走向凋亡

01 Cell culture and experimental setup



02 Cell counting

trypsin-digested

Trypan blue

hemocytometer

03 DNA staining and flow cytometry

trypsin-digested

single-cell suspensions -DPBS

fixed and permeabilized

rehydrated

RNase A -digest cellular RNA

propidium iodide (PI)

flow cytometry analysis

03

Results

Stress treatment triggers a variety of responses across time

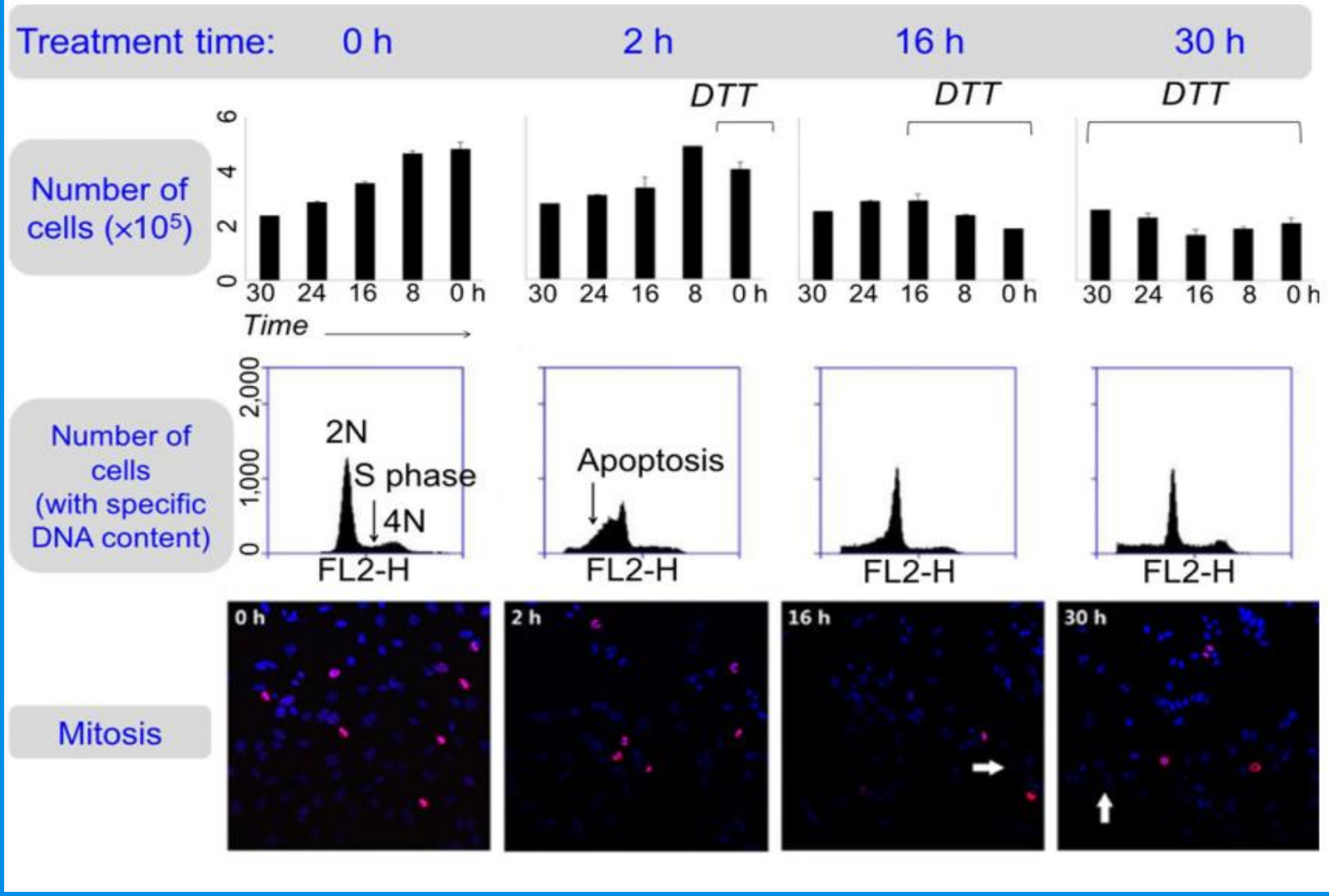
The integrated transcriptome and proteome are highly dynamic

A statistical tool identifies hidden regulatory signals

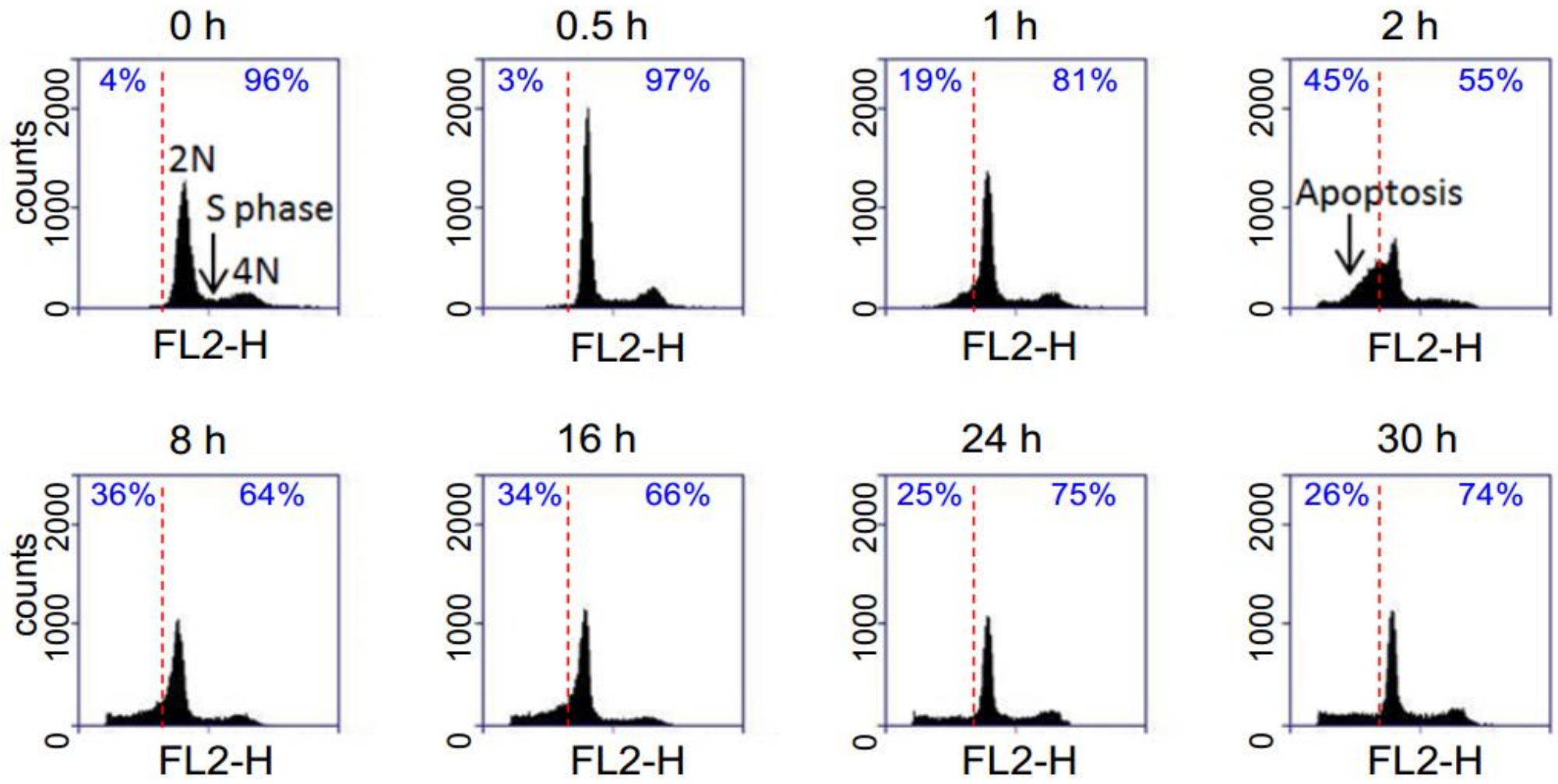
Protein concentration changes occur in greater magnitude, but both regulatory levels contribute equally and independently

Protein expression regulation reaches a new steady state

PECA results help to generate hypotheses on regulatory modes

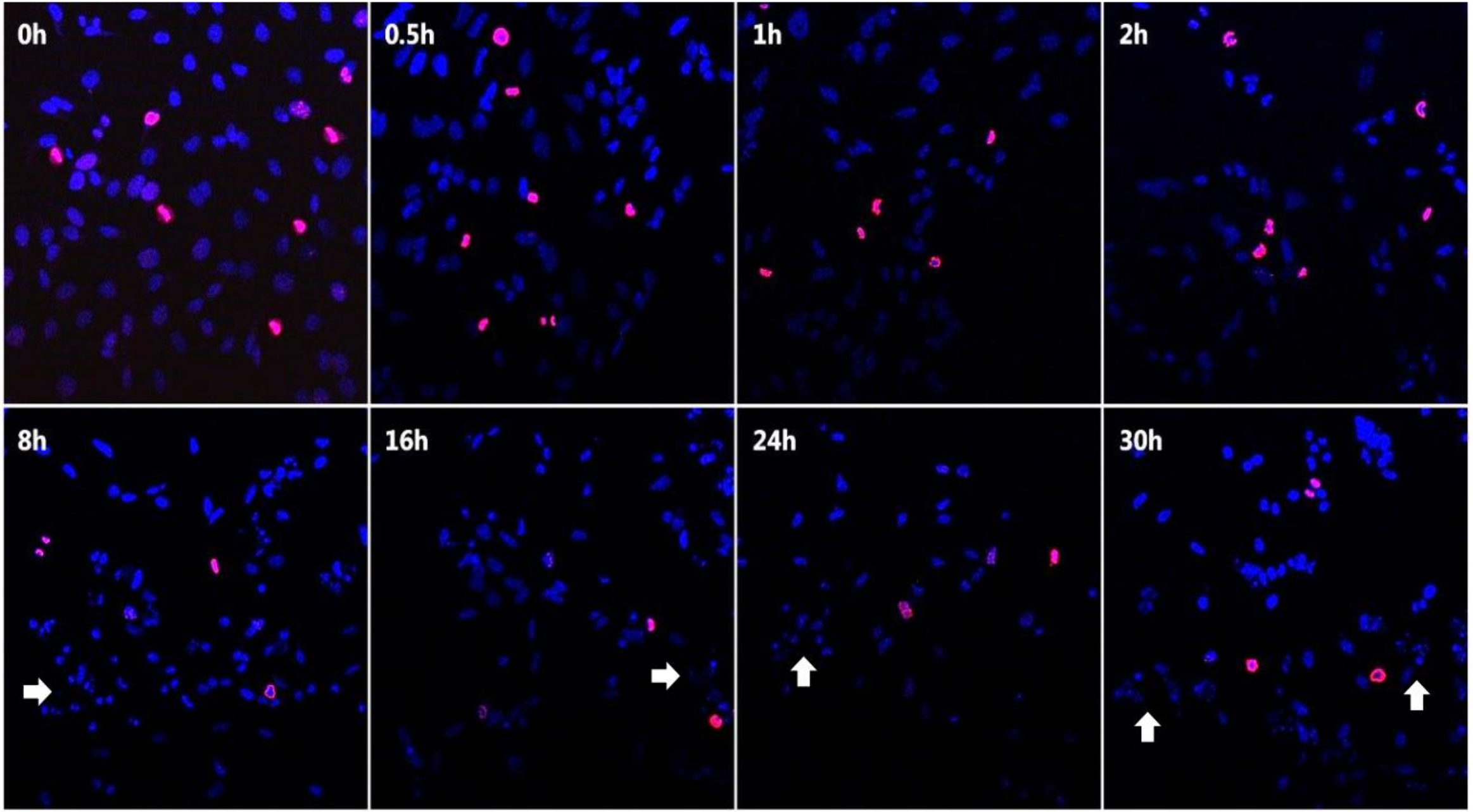

Treatment time: 0 h 2 h 16 h 30 h
DTT
DTT
DTT
Number of cells (×10^5)
0
2
4
6
30 24 16 8 0 h
30 24 16 8 0 h
30 24 16 8 0 h
30 24 16 8 0 h
Time
Number of cells (with specific DNA content)
0
1,000
2,000
2N
S phase
4N
Apoptosis
FL2-H
FL2-H
FL2-H
FL2-H
Mitosis
0 h
2 h
16 h
30 h

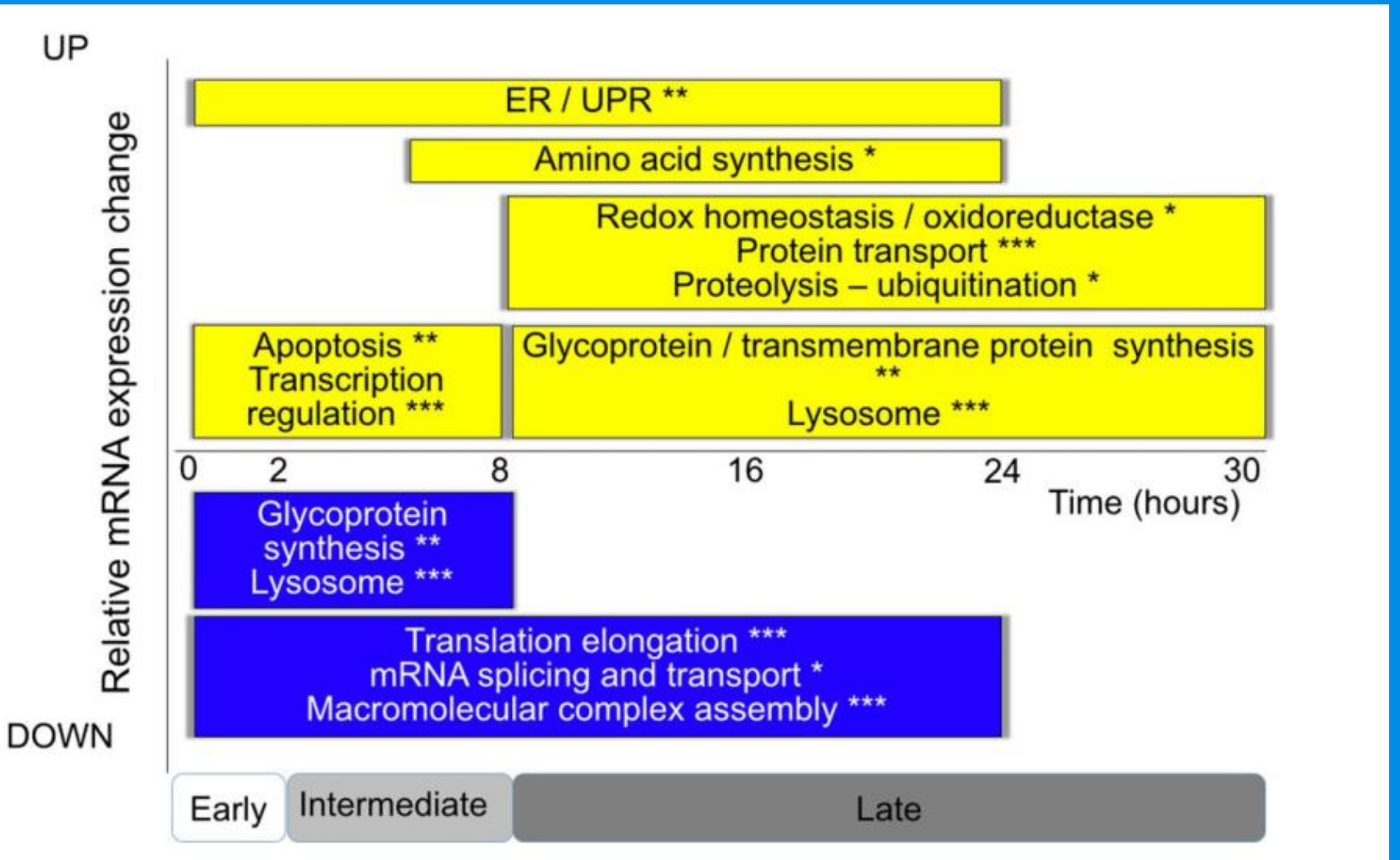
A.

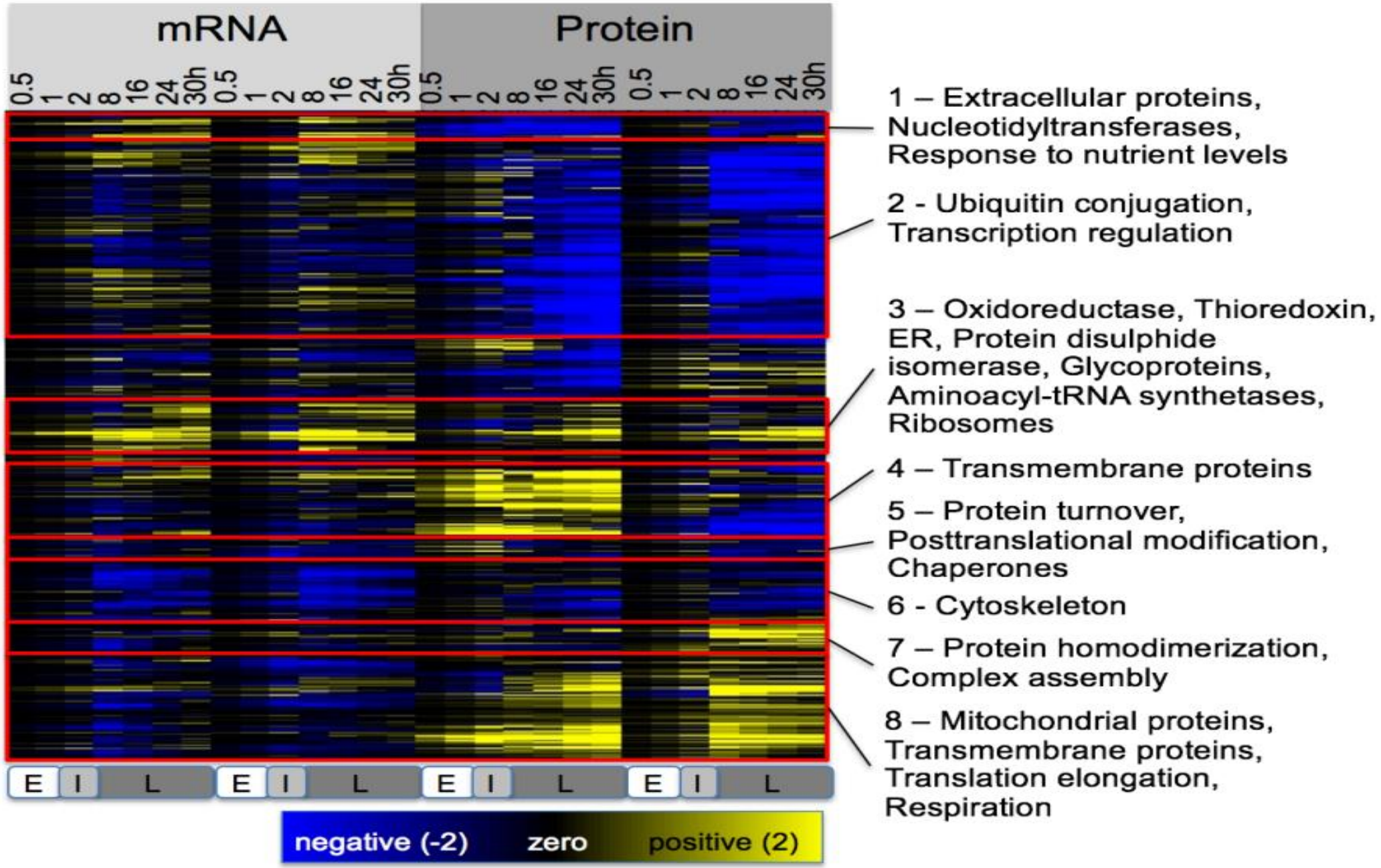


Key: *Percentage cells (area)* ------ *Apoptotic (left) and non-apoptotic cells (right)*

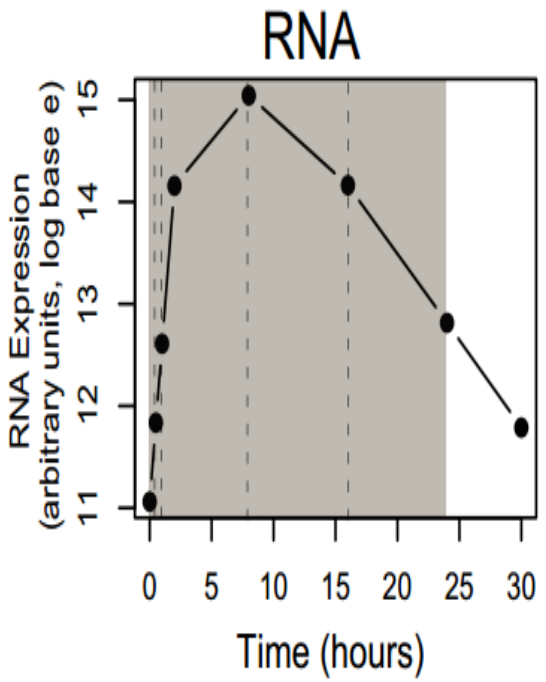
B.

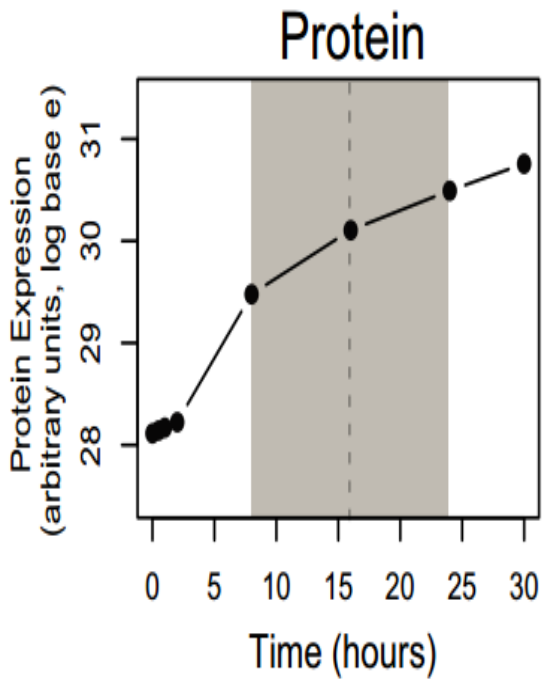



UP
Relative mRNA expression change
DOWN
ER / UPR **
Amino acid synthesis *
Redox homeostasis / oxidoreductase *
Protein transport ***
Proteolysis – ubiquitination *
Apoptosis **
Transcription regulation ***
Glycoprotein / transmembrane protein synthesis **
Lysosome ***
0
2
8
16
24
30
Time (hours)
Glycoprotein synthesis **
Lysosome ***
Translation elongation ***
mRNA splicing and transport *
Macromolecular complex assembly ***
Early
Intermediate
Late

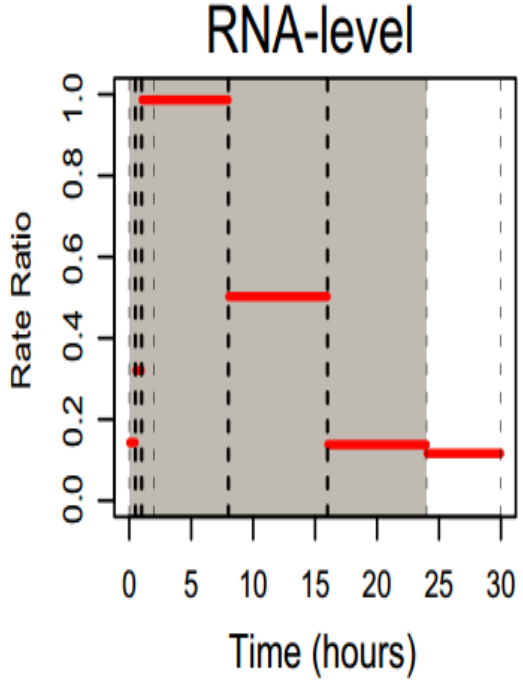

mRNA
Protein
0.5
1
2
8
16
24
30h
E
I
L
negative (-2)
zero
positive (2)
1 – Extracellular proteins, Nucleotidyltransferases, Response to nutrient levels
2 - Ubiquitin conjugation, Transcription regulation
3 – Oxidoreductase, Thioredoxin, ER, Protein disulphide isomerase, Glycoproteins, Aminoacyl-tRNA synthetases, Ribosomes
4 – Transmembrane proteins
5 – Protein turnover, Posttranslational modification, Chaperones
6 - Cytoskeleton
7 – Protein homodimerization, Complex assembly
8 – Mitochondrial proteins, Transmembrane proteins, Translation elongation, Respiration

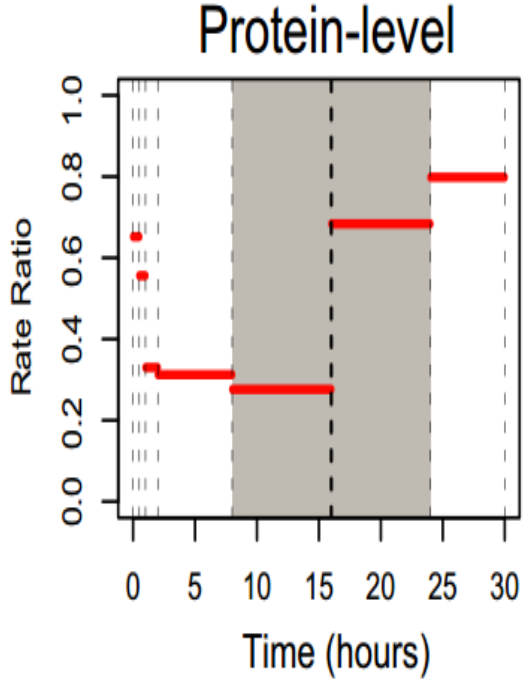
Concentration



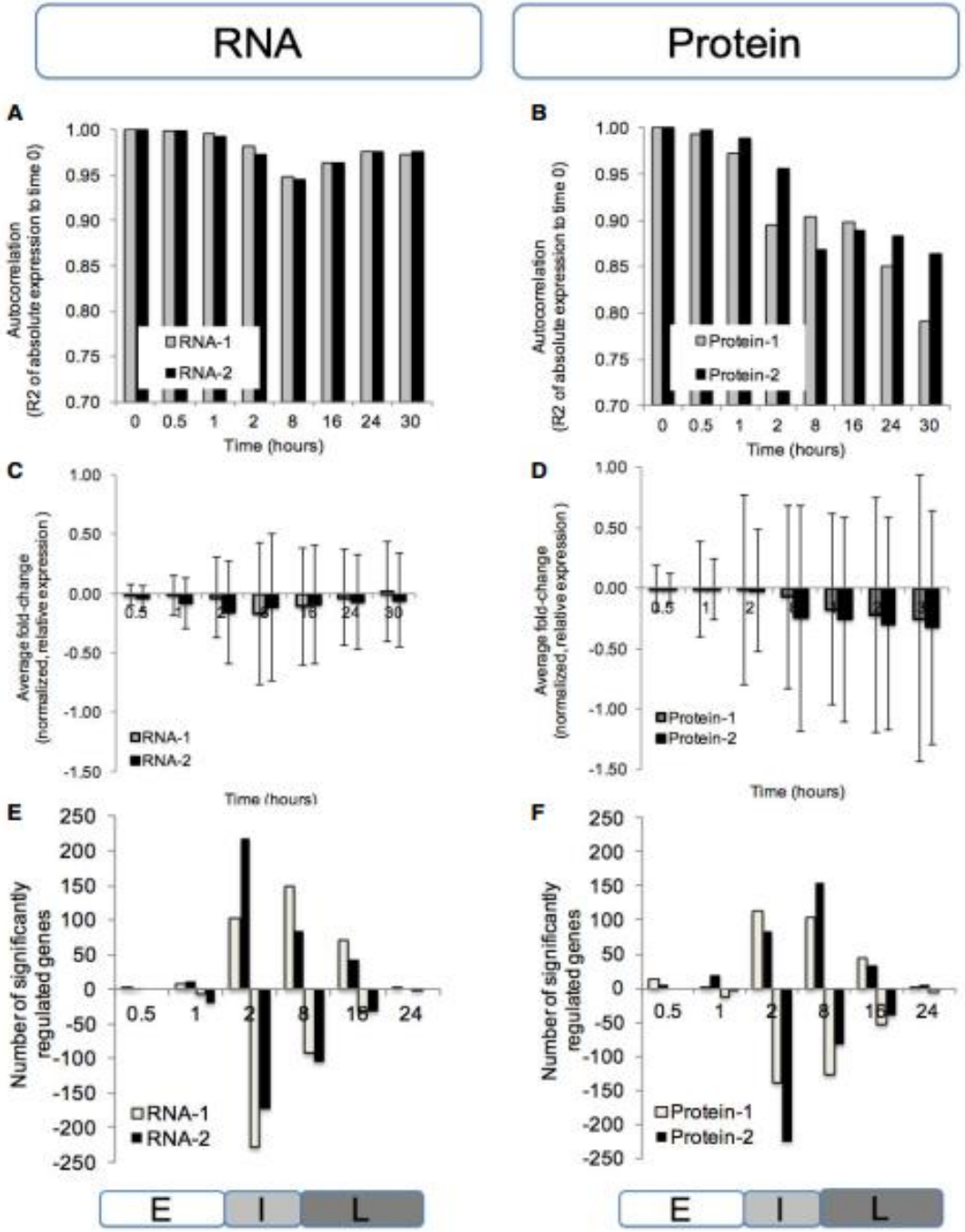


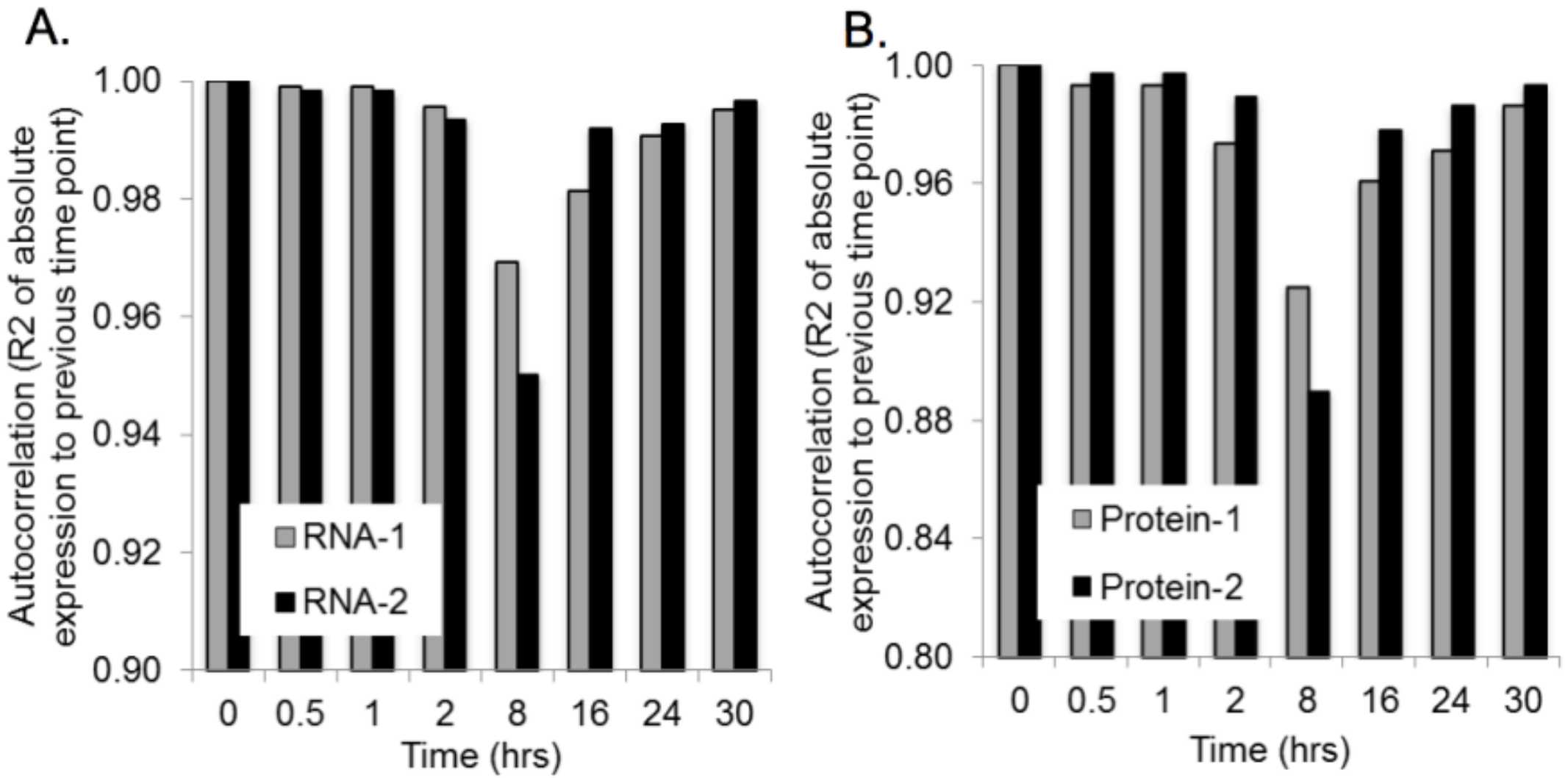
Regulation analysis (PECA)

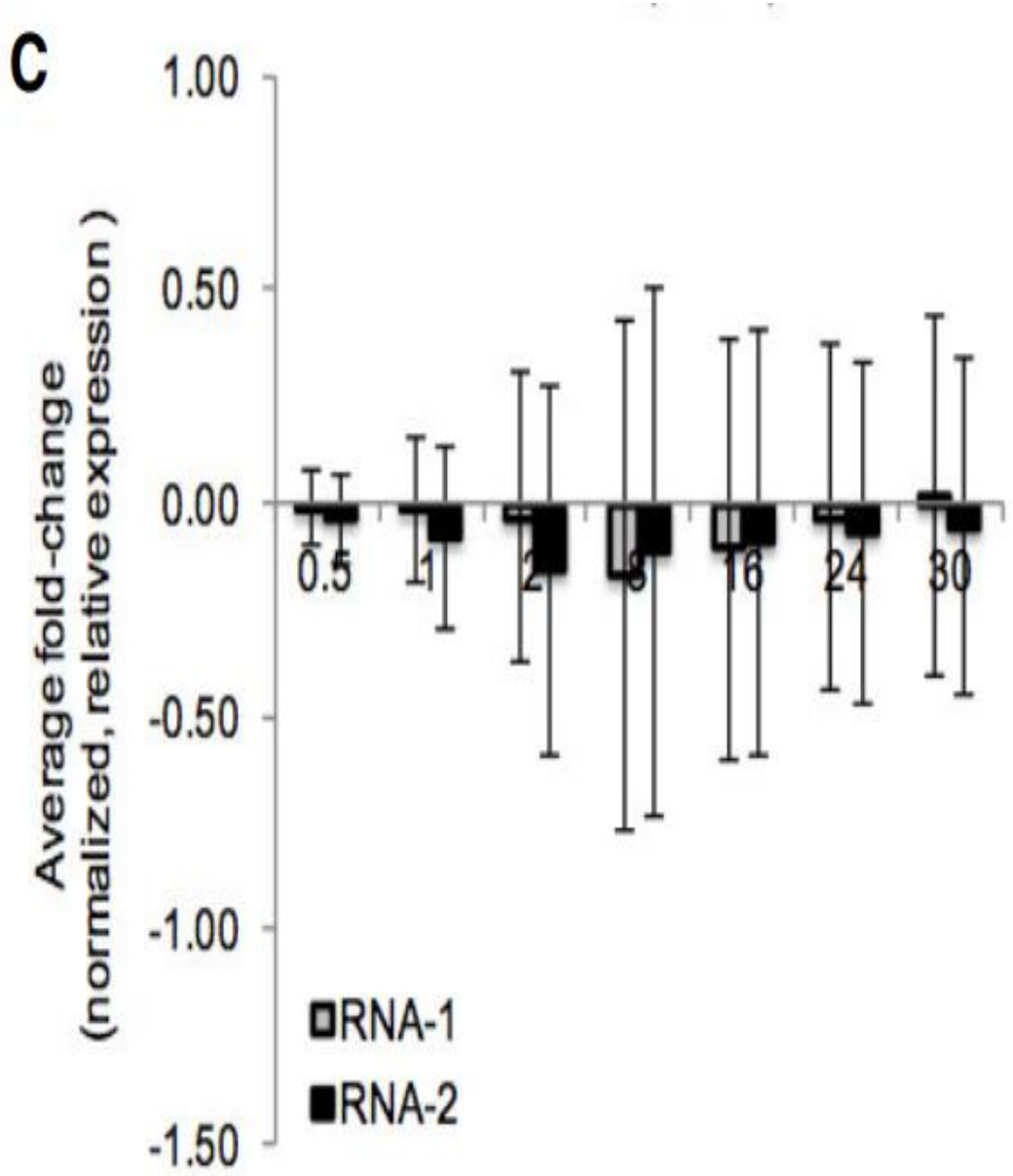


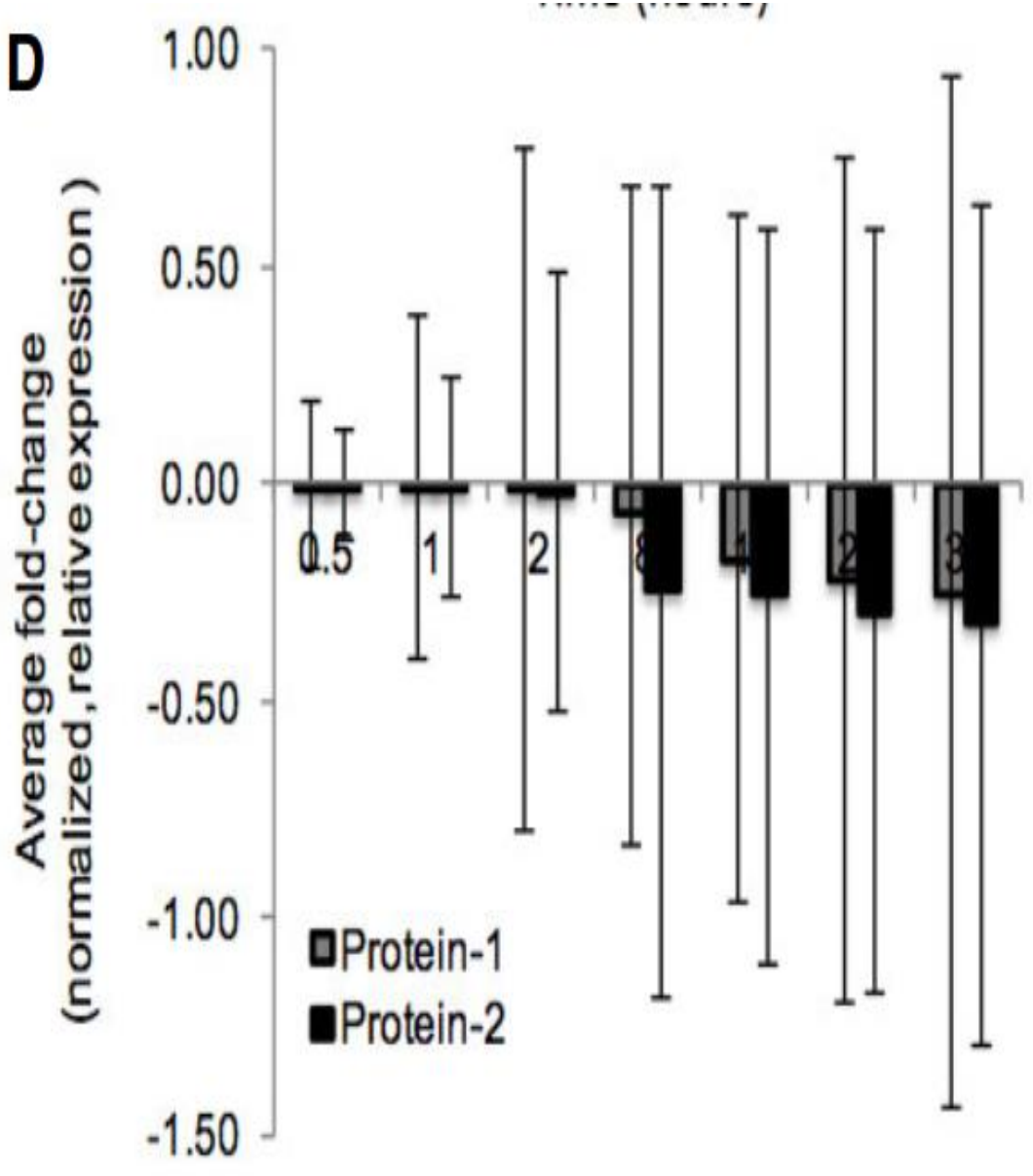


Grey: significant regulation (FDR<0.05)


RNA
Protein
A
B
C
D
E
F
Autocorrelation (R2 of absolute expression to time 0)
Average fold-change (normalized, relative expression)
Number of significantly regulated genes
Time (hours)
RNA-1
RNA-2
Protein-1
Protein-2
E
I
L


A.
Autocorrelation (R2 of absolute expression to previous time point)
1.00
0.98
0.96
0.94
0.92
0.90
RNA-1
RNA-2
0
0.5
1
2
8
16
24
30
Time (hrs)
B.
Autocorrelation (R2 of absolute expression to previous time point)
1.00
0.96
0.92
0.88
0.84
0.80
Protein-1
Protein-2
0
0.5
1
2
8
16
24
30
Time (hrs)


C
1.00
0.50
0.00
-0.50
-1.00
-1.50
Average fold-change
(normalized, relative expression)
0.5
1
2
8
16
24
30
RNA-1
RNA-2


D
1.00
0.50
0.00
-0.50
-1.00
-1.50
Average fold-change
(normalized, relative expression)
0.5
1
2
Protein-1
Protein-2

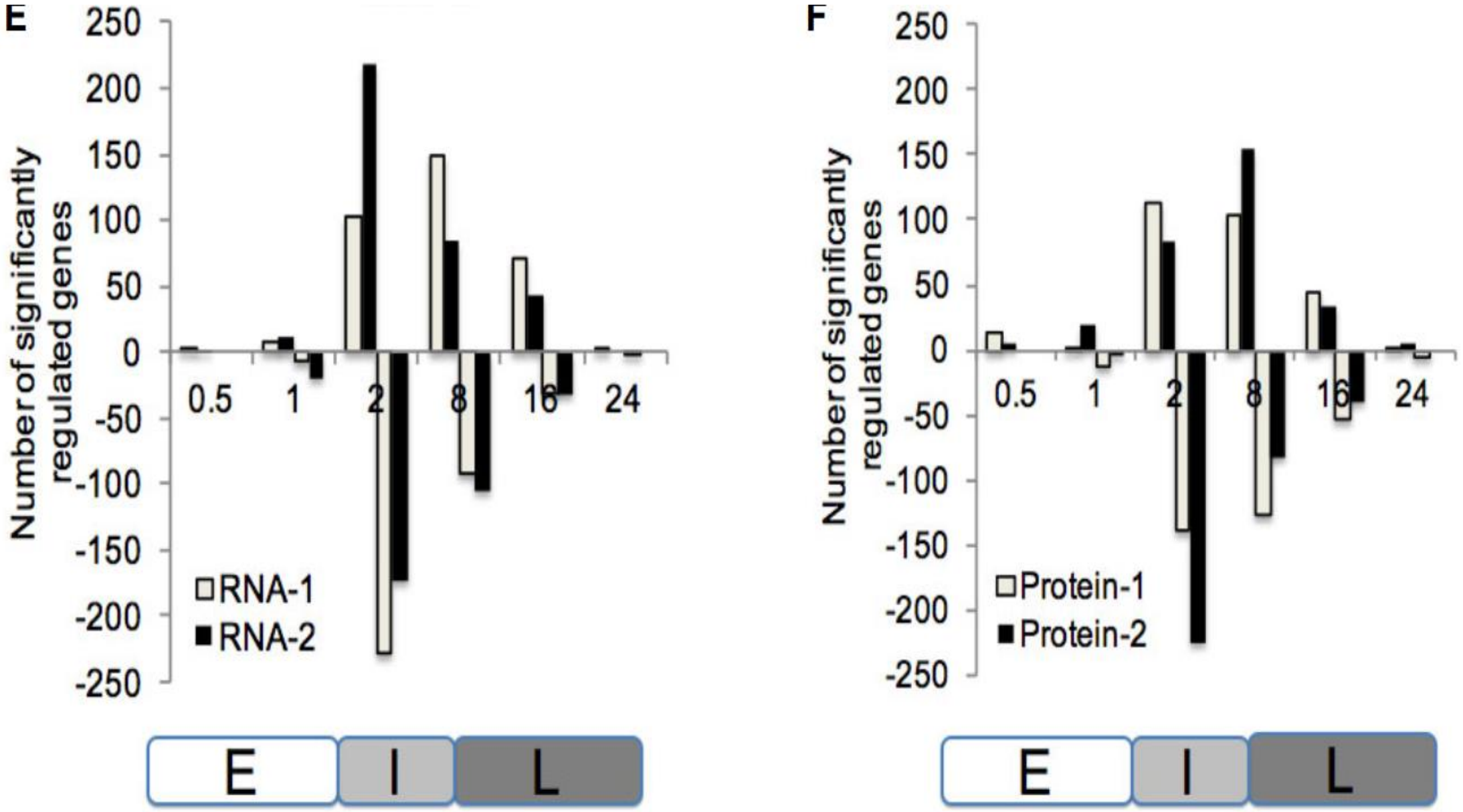
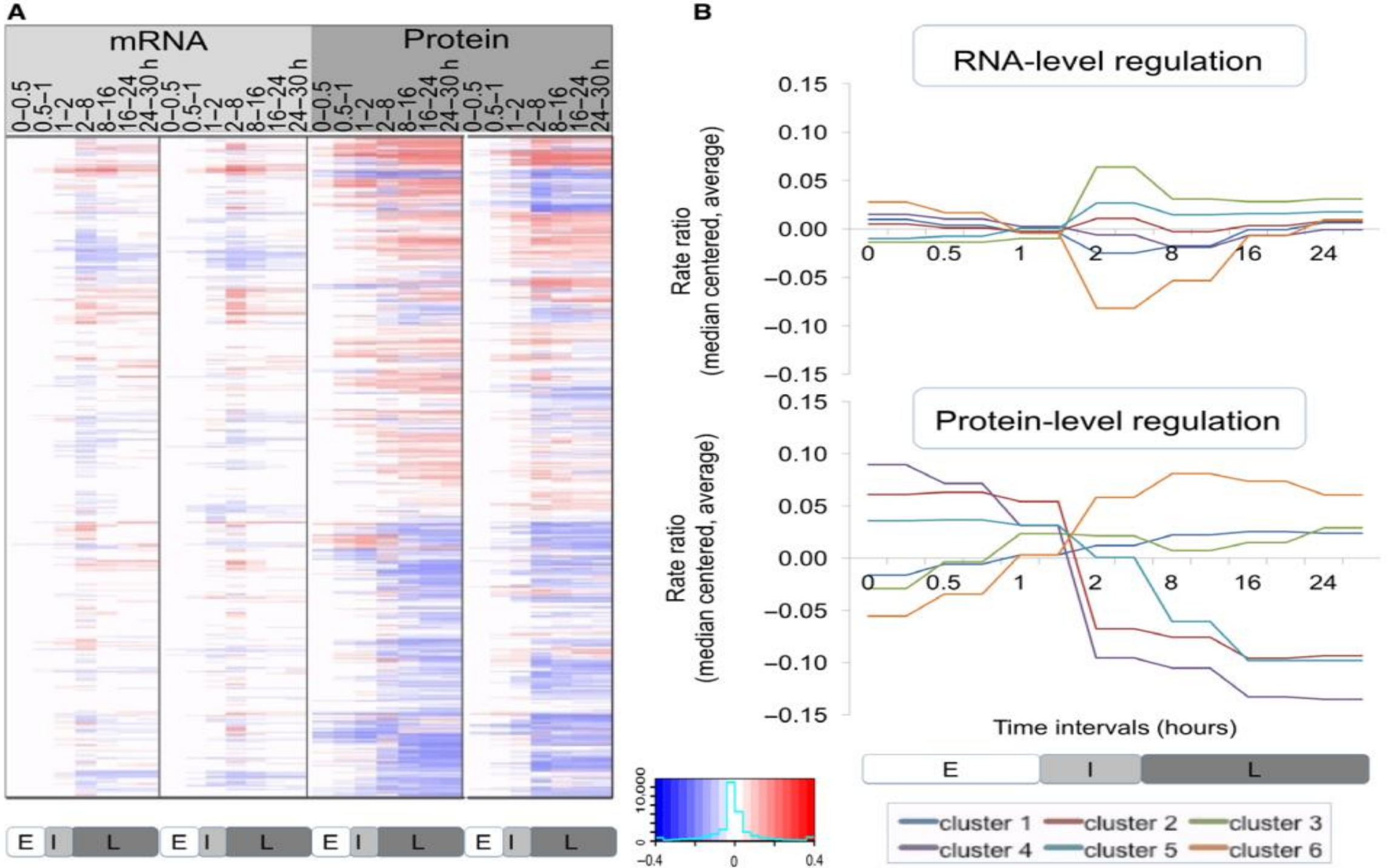

E
250
200
150
100
50
0
-50
-100
-150
-200
-250
Number of significantly regulated genes
0.5
1
2
8
16
24
RNA-1
RNA-2
E
I
L
F
250
200
150
100
50
0
-50
-100
-150
-200
-250
Number of significantly regulated genes
0.5
1
2
8
16
24
Protein-1
Protein-2
E
I
L

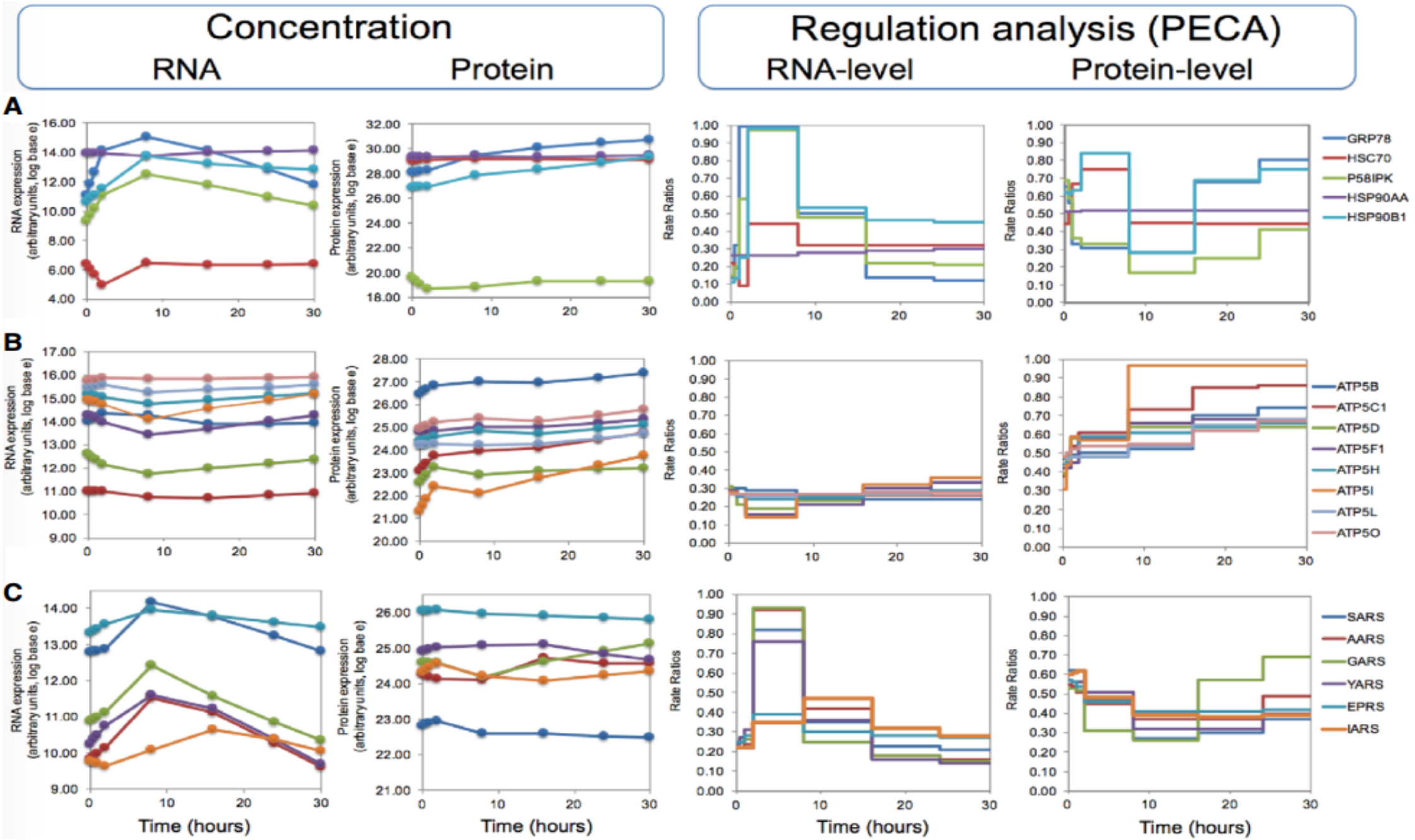
Table 1. RNA- and protein-level regulations contribute equally to gene expression.

	Early			Intermediate			Late		
Protein / RNA	Down	None	Up	Down	None	Up	Down	None	Up
Down	0	7	0	26	180	21	35	63	16
None	13	1194	15	99	726	83	116	728	94
Up	0	8	0	15	77	10	23	138	24

Scale
>500
>200
>50
>20
>0

Using PECA, we extracted genes that are significantly regulated at the RNA level, the protein level, at both levels, or neither (FDR < 0.05). The tables group these genes into the three different phases ("early", "intermediate", and "late") and distinguish between up- and down-regulation, marked by "Up" and "Down", respectively. Most changes occur during the intermediate phase. The distribution of the numbers across the tables is symmetric, indicating that mRNA- and protein-level regulations are equally important.


A
mRNA
Protein
0–0.5
0.5–1
1–2
2–8
8–16
16–24
24–30 h
E
I
L
B
RNA-level regulation
Protein-level regulation
Rate ratio
(median centered, average)
0.15
0.10
0.05
0.00
−0.05
−0.10
−0.15
0
0.5
1
2
8
16
24
Time intervals (hours)
10,000
−0.4
0
0.4
cluster 1
cluster 2
cluster 3
cluster 4
cluster 5
cluster 6


Concentration
RNA
Protein
Regulation analysis (PECA)
RNA-level
Protein-level
A
B
C
RNA expression (arbitrary units, log base e)
Protein expression (arbitrary units, log base e)
Rate Ratios
Time (hours)
GRP78
HSC70
P58IPK
HSP90AA
HSP90B1
ATP5B
ATP5C1
ATP5D
ATP5F1
ATP5H
ATP5I
ATP5L
ATP5O
SARS
AARS
GARS
YARS
EPRS
IARS

04

Personal summary

1、实验设计非常完美

2、数据处理有理有据

3、结果分析全面细致

蛋白水平与mRNA水平调控同等重要但存在差异

THANK YOU!