A Combination Approach of Pseudotime Analysis and Mathematical Modeling for Understanding Drug-Resistant Mechanisms

Roberto Pagliarini

Aim

- Elucidating the molecular mechanisms underlying the process of drug resistance acquisition

  - Sequential analysis of gene expression patterns in Tamoxifen (TAM)-treated breast cancer cells

    - Bulk RNA-sequencing (RNA-seq)
    - Single-cell RNA-sequencing
    - Matemathical modeling

Aim → Main results

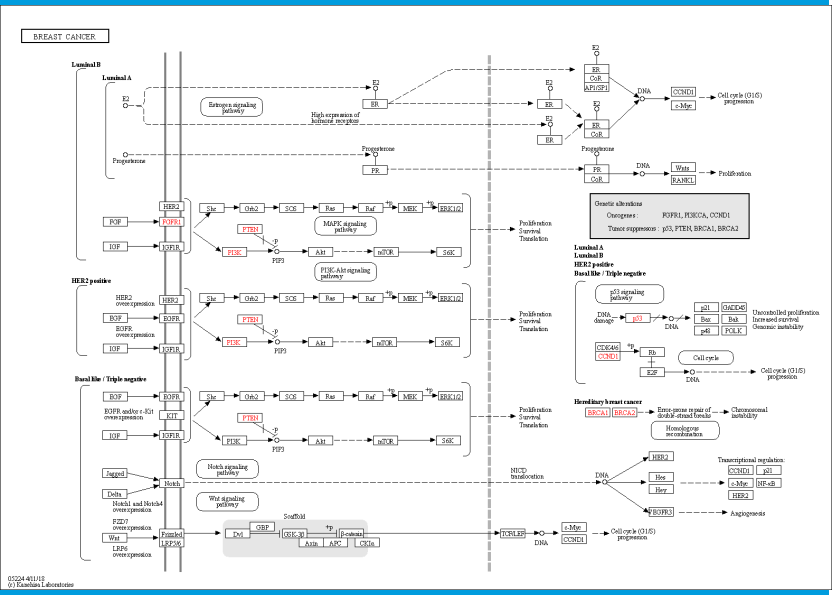
- Sequential analysis of gene expression patterns in Tamoxifen (TAM)-treated breast cancer cells

  - TAM-resistant cells can be divided in two groups
    - One showing altered gene-expression related to metabolic regulation
    - One showing high expression levels of adhension-related molecules and histone-modiyfing enzimes

  - Cell transition trajectory to the two resistant groups that derived from a shared pre-resistant state

  - Mathematical modeling predicts that inhibition of transition to both resistant subtypes would prevent the TAM resistance

Estrogen Receptor (ER)



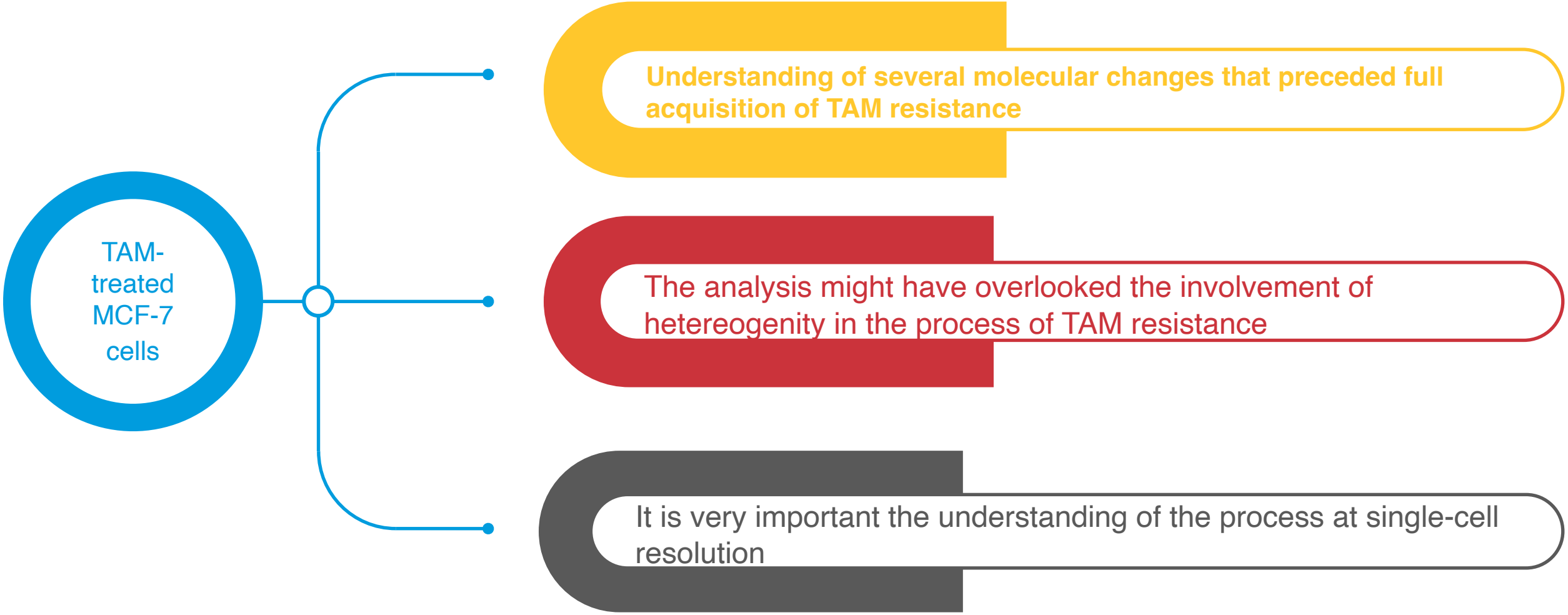
1) It is a hormone-dependent TF that plays an important role in many physiological process

2) ER is closely associated with breast cancer develeopment

3) 75% of all breast cancer cases are ER-positive luminal subtypes and initially treated with Tamoxifen, an ER antagonist

4) 40% of TAM-responsive tumors progress to resistant and metastatic tumors after long-treatment

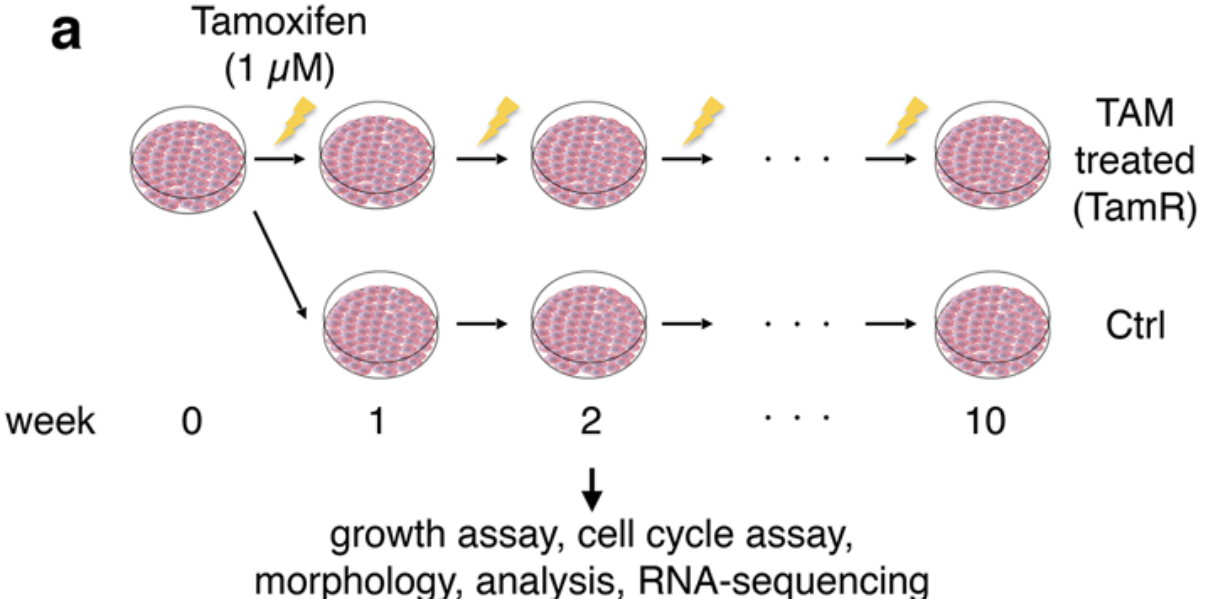
Bulk RNA-sequencing of a human ER-positive breast cancer cell line



Time-series transcriptome profiles of MCF-7 cells during continuous TAM treatment

Result 1

Overview of experimental procedure

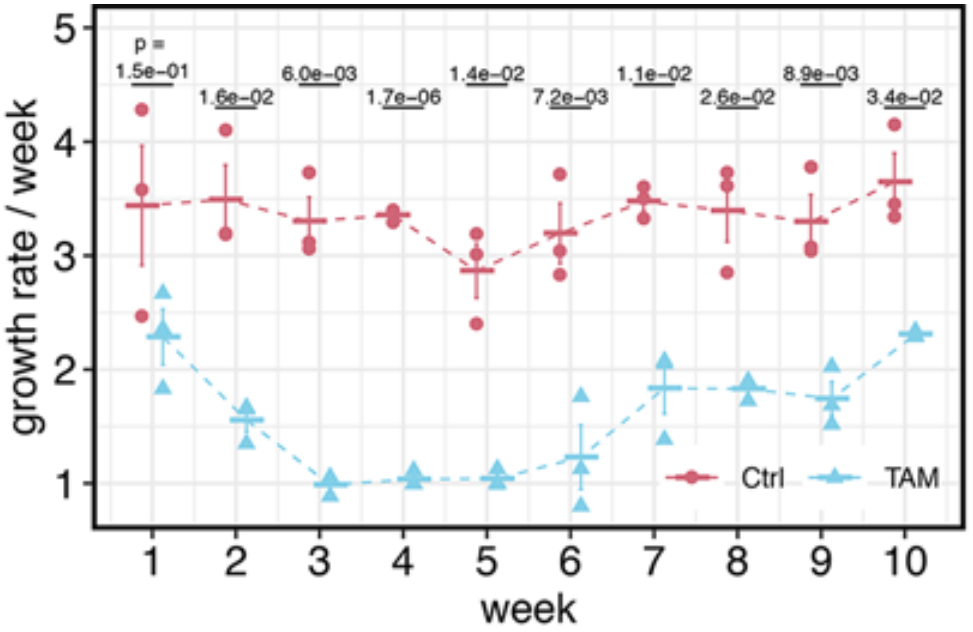


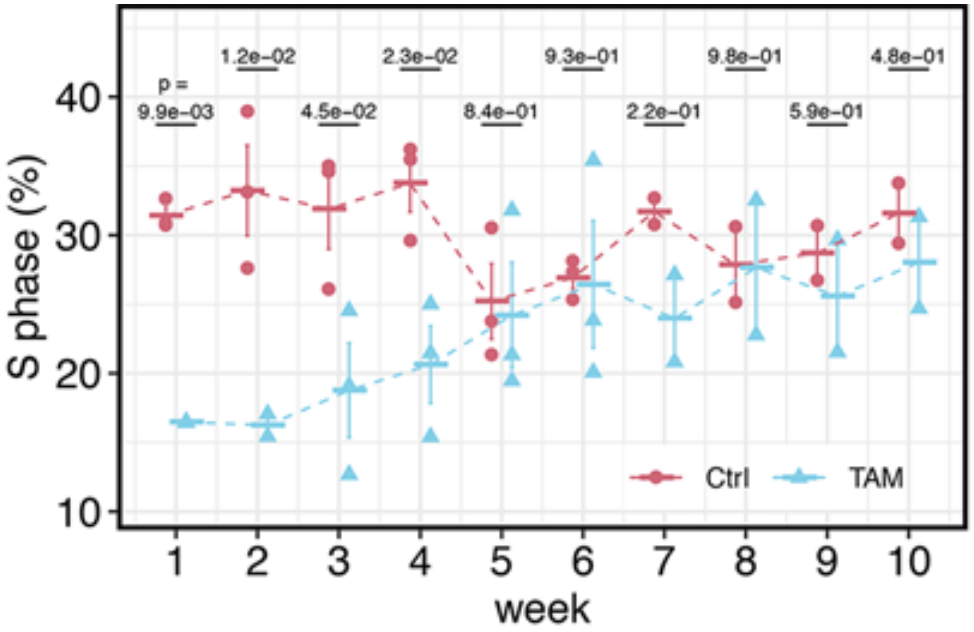
1) Human breast adenocarcinoma MCF-7 cells

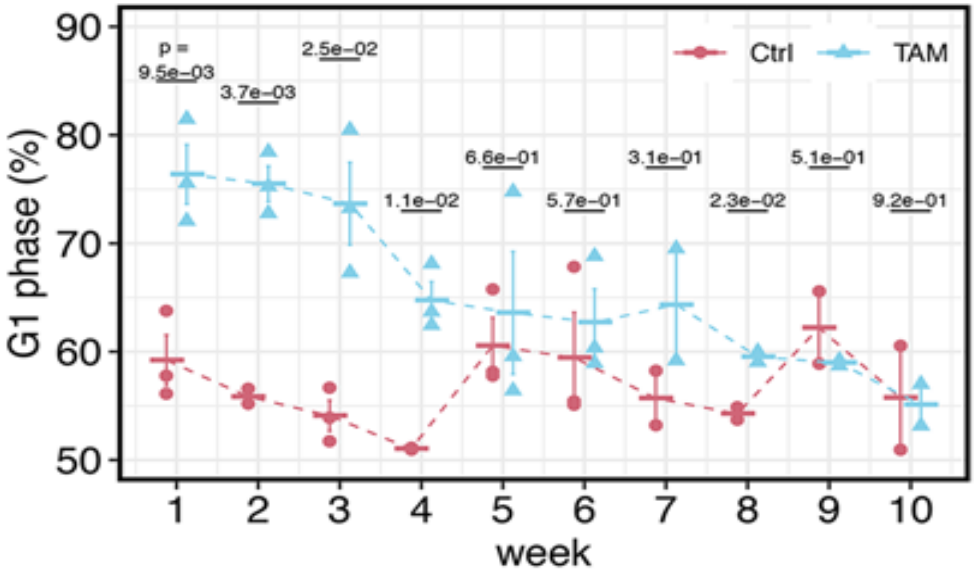
2) Cultured medium supplemented with or without TAM

3) Every week, cells were detached and collected with trypsinization for the analysis

TAM treatment initially inhibited cell growth by decreasing the number of cells in S phase and increasing that in G1 phase





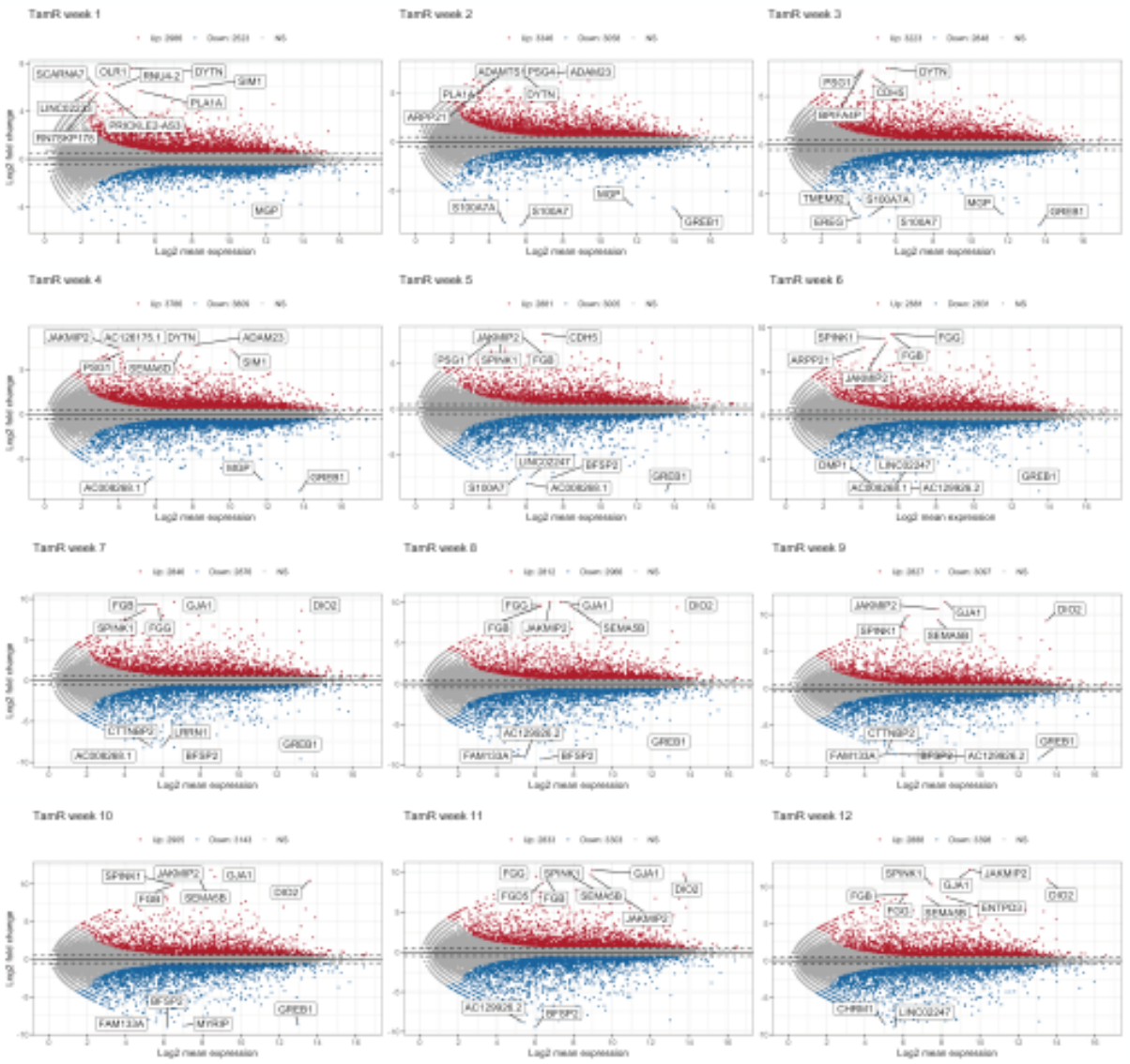


1) The growth of cells were completely inhibited until W5

2) The cell-cycle of TAM-treated cells was dysregulated until W5

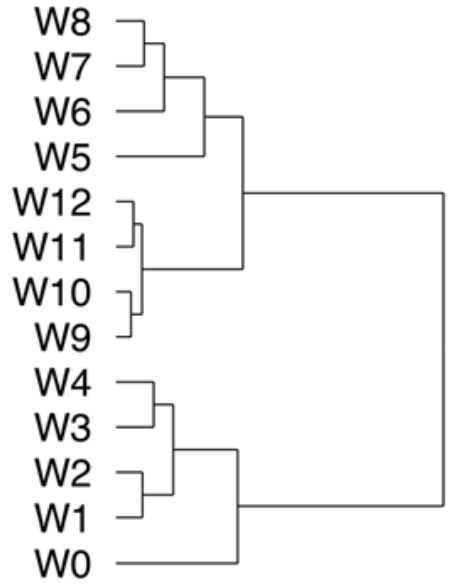
3) This result showed the process by which the cells survive and restored their growth potential in the absence of ER signaling

Bulk RNA-seq data of TAM-treated MCF-7 cells



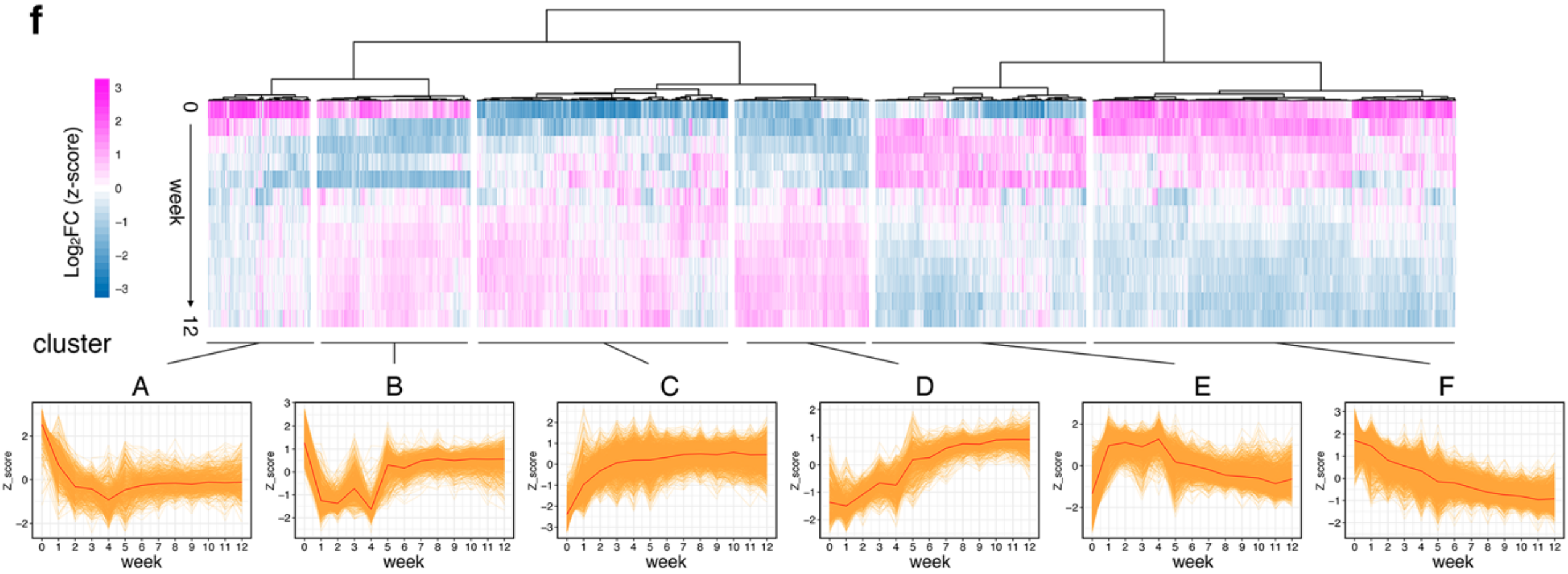
Week	1	2	3	4	5	6	7	8	9	10	11	12
DEGs (Up)	2966	3346	3223	3786	2881	2851	2846	2812	2827	2905	2833	2688
DEGs (Down)	2523	3058	2848	3609	3005	2831	2676	2966	2097	3143	3303	3398

≈ 7000 DEGs

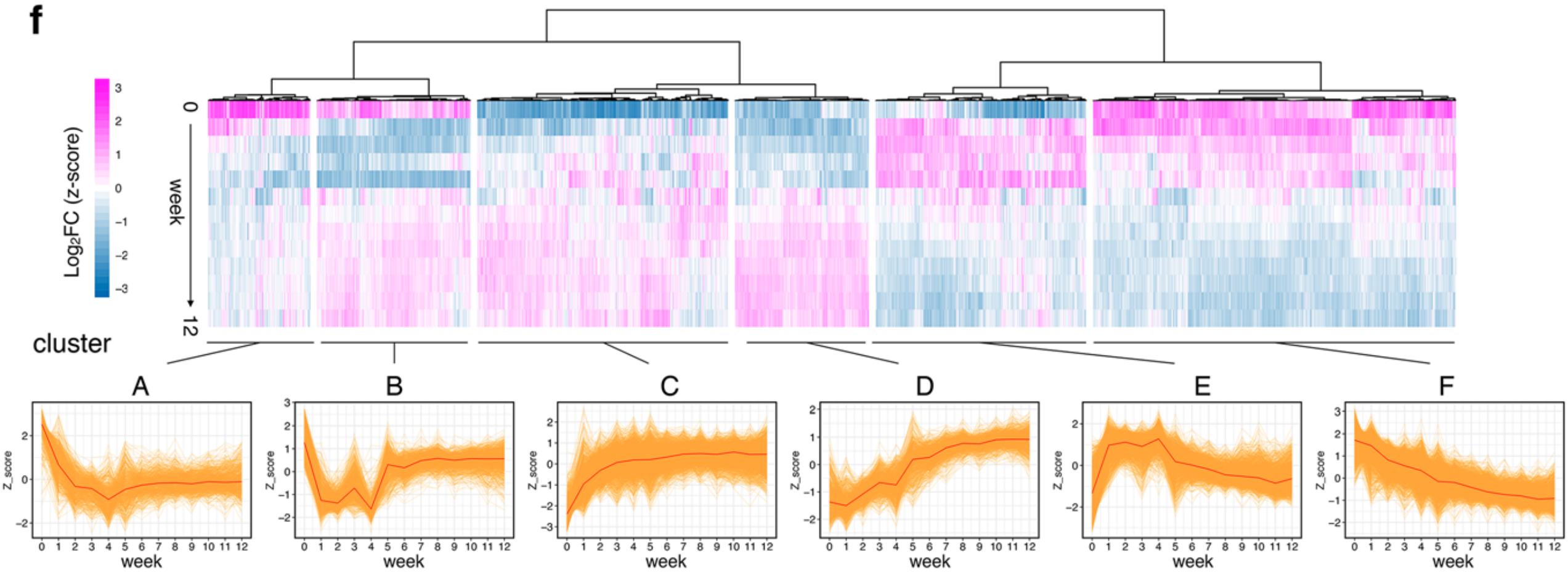


This result indicates that DEG expression patterns became stable at the later stage

DEGs cluster analysis

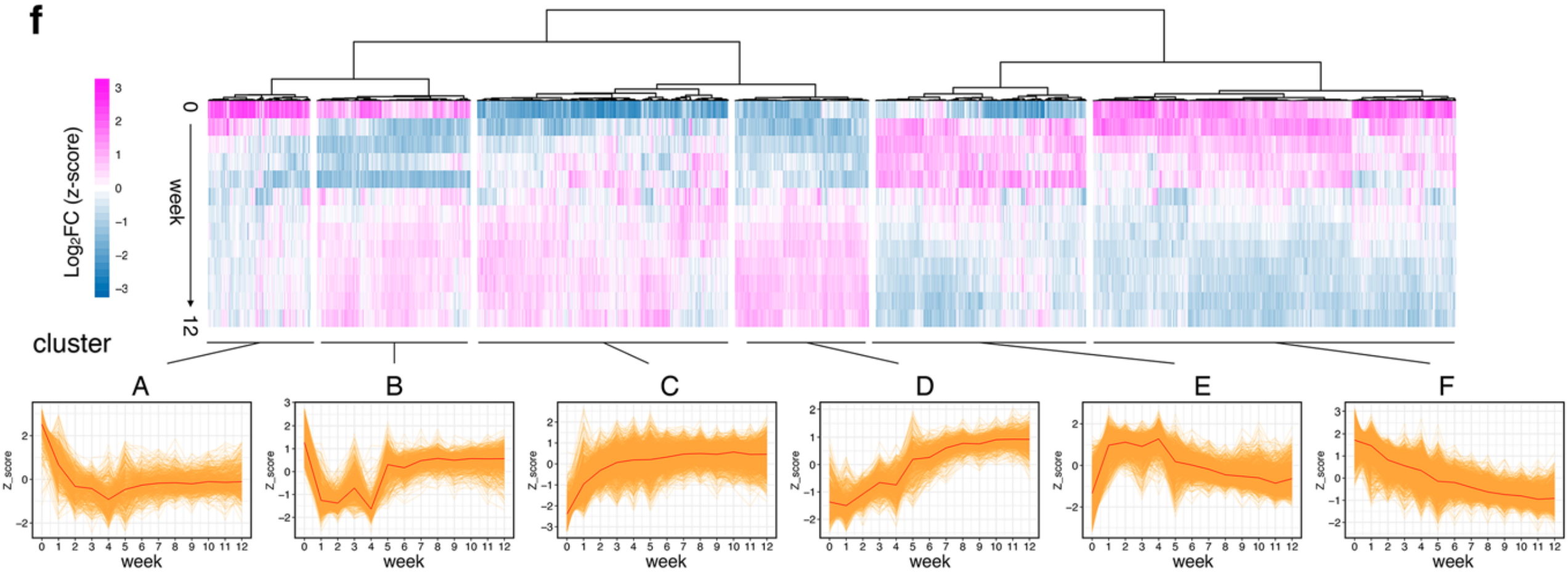


DEGs cluster analysis



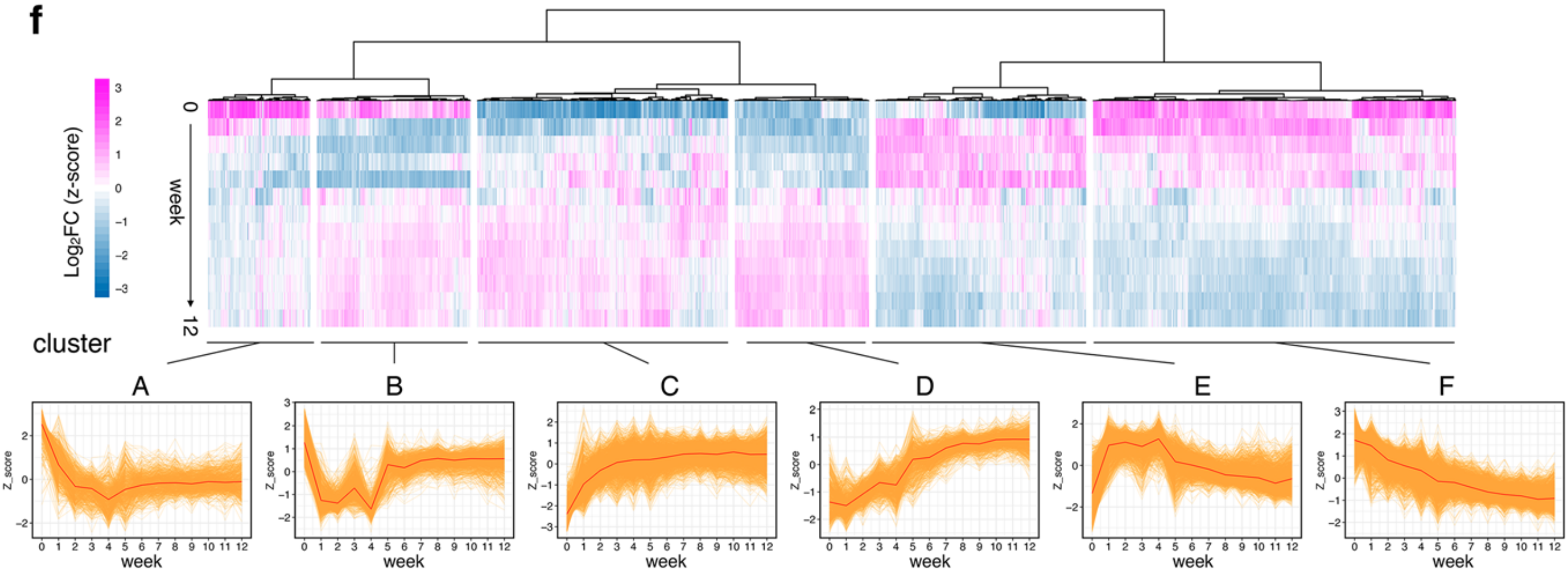
Cluster A: rapidly decreasing expression

DEGs cluster analysis



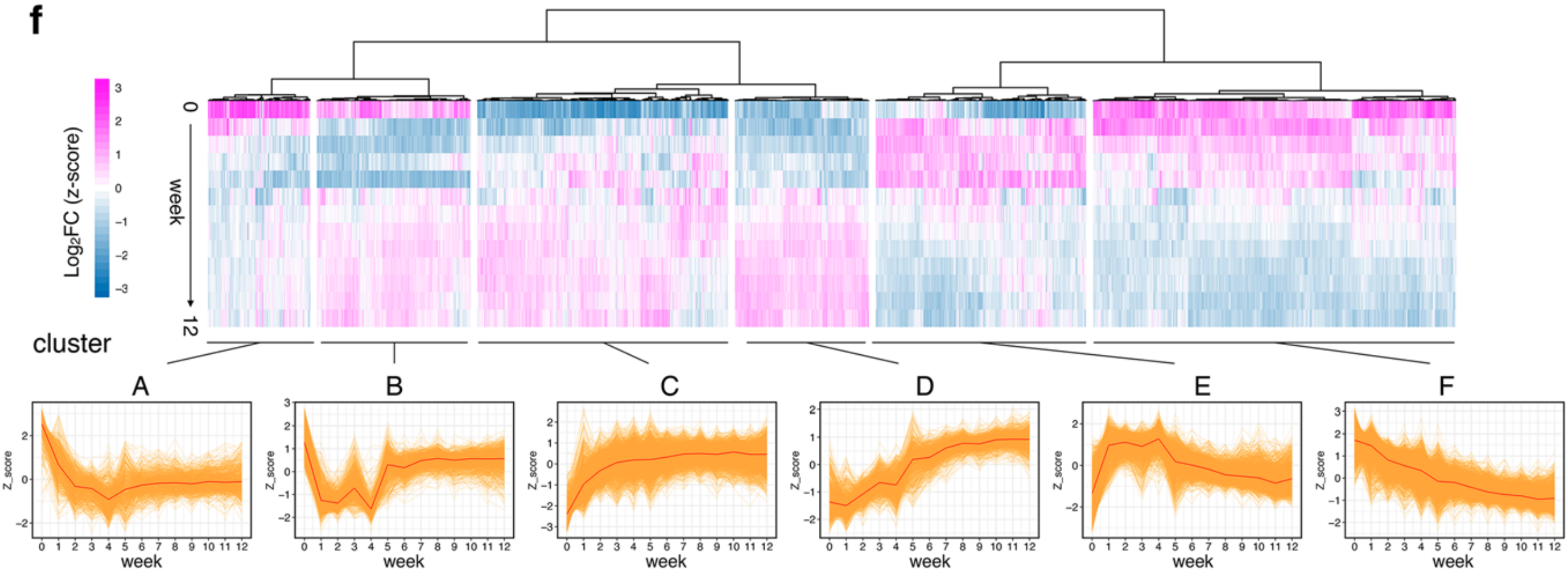
Cluster B: initially down-regulated, and recovered at W5

DEGs cluster analysis



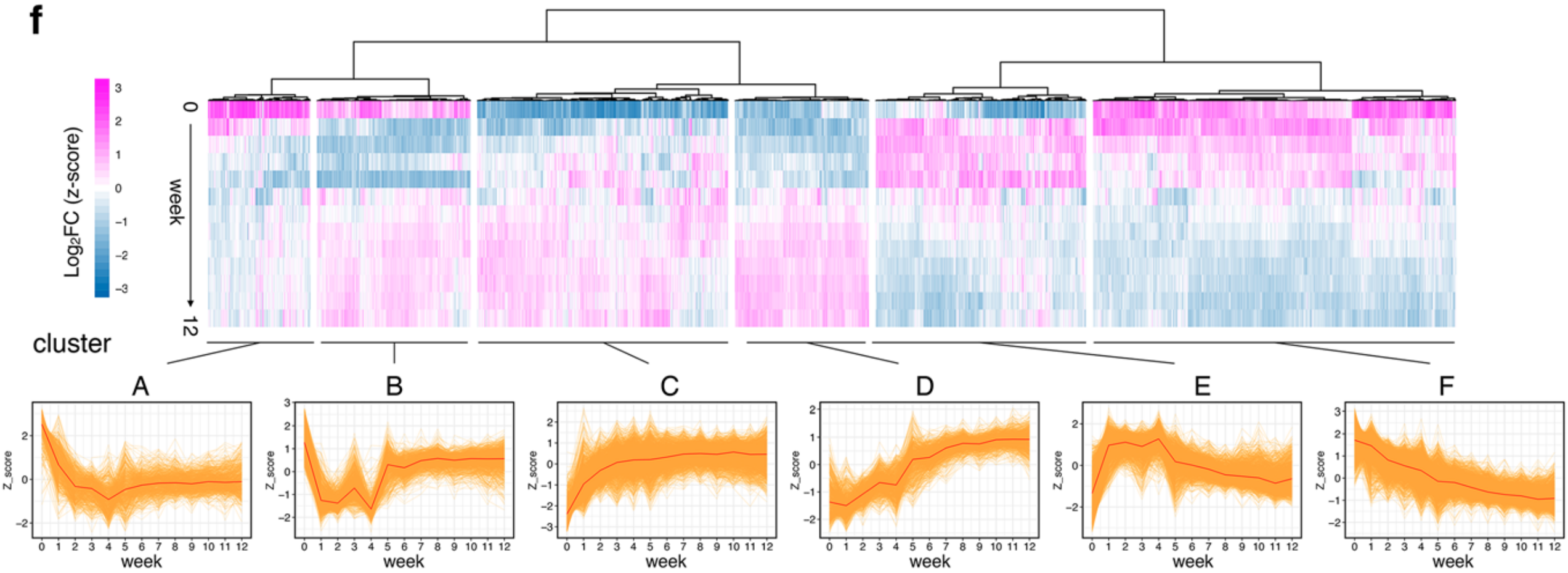
Cluster C: rapidly increasing before cell growth rate recovery

DEGs cluster analysis



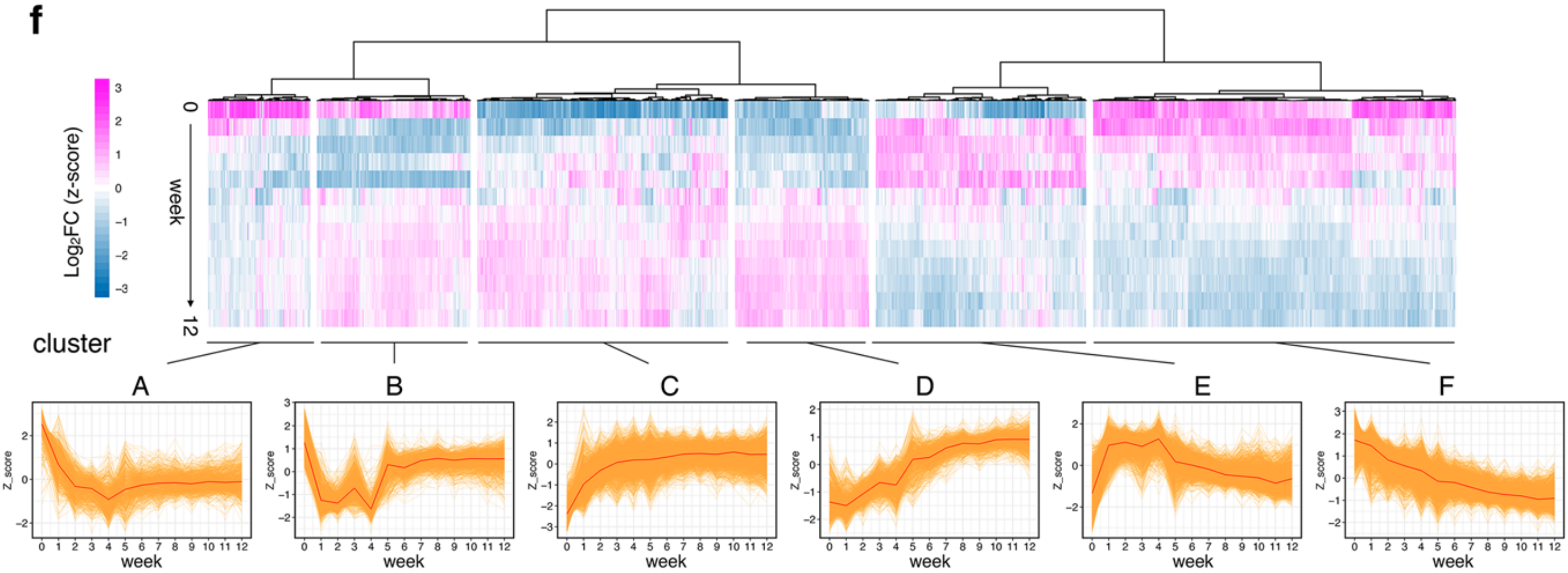
Cluster D: a gradual increase in expression concomitant with growt rate recovery

DEGs cluster analysis



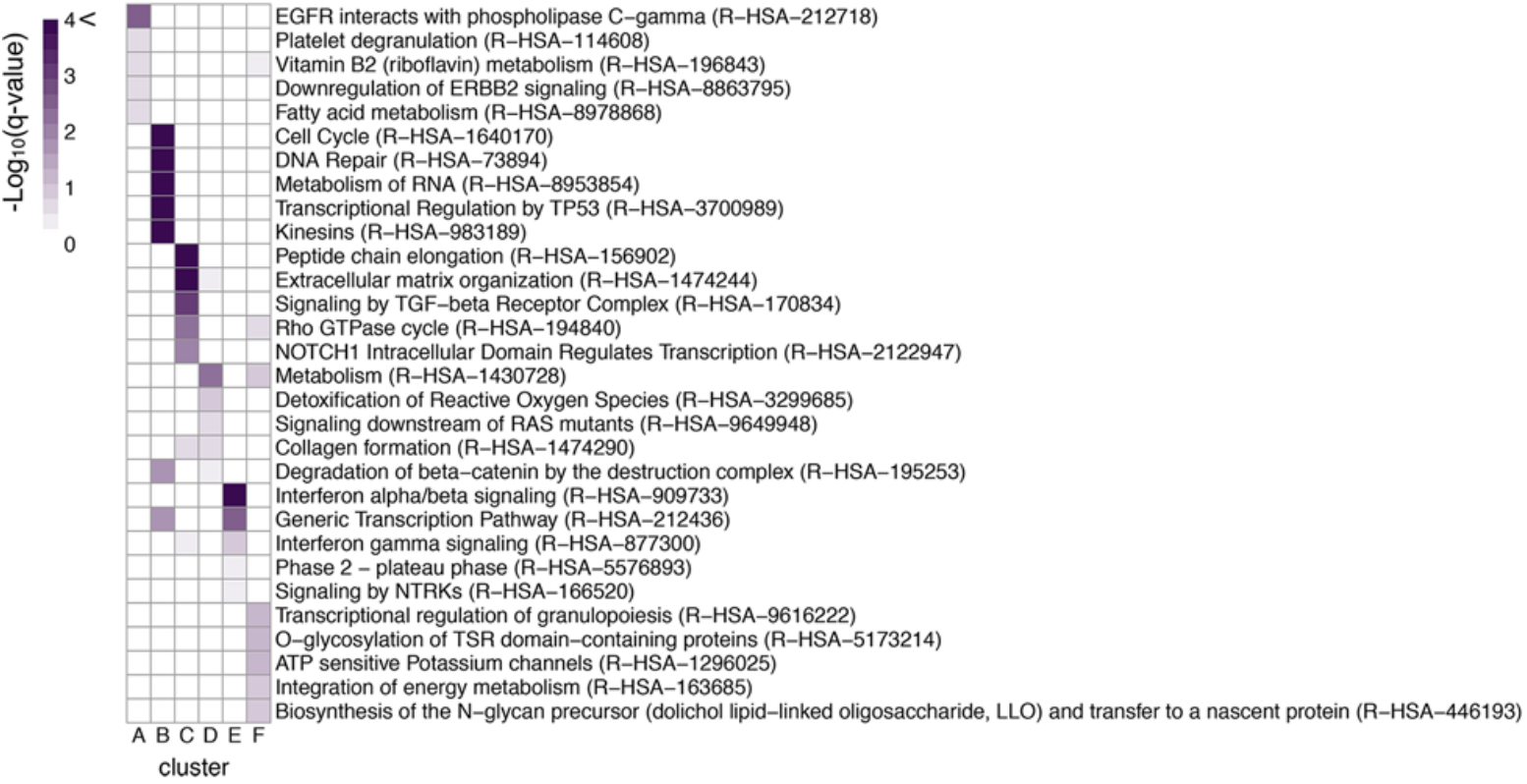
Cluster E: initially up-regulated, and then down-regulated

DEGs cluster analysis



Cluster F: gradually decreasing

Clusters Enrichment Analysis



Reactome pathways database

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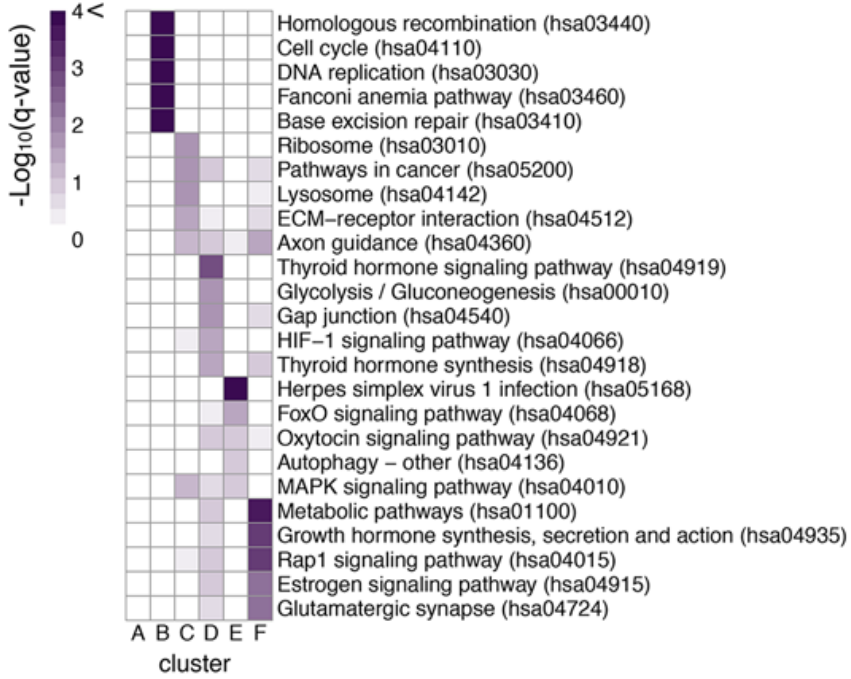
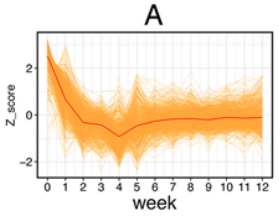
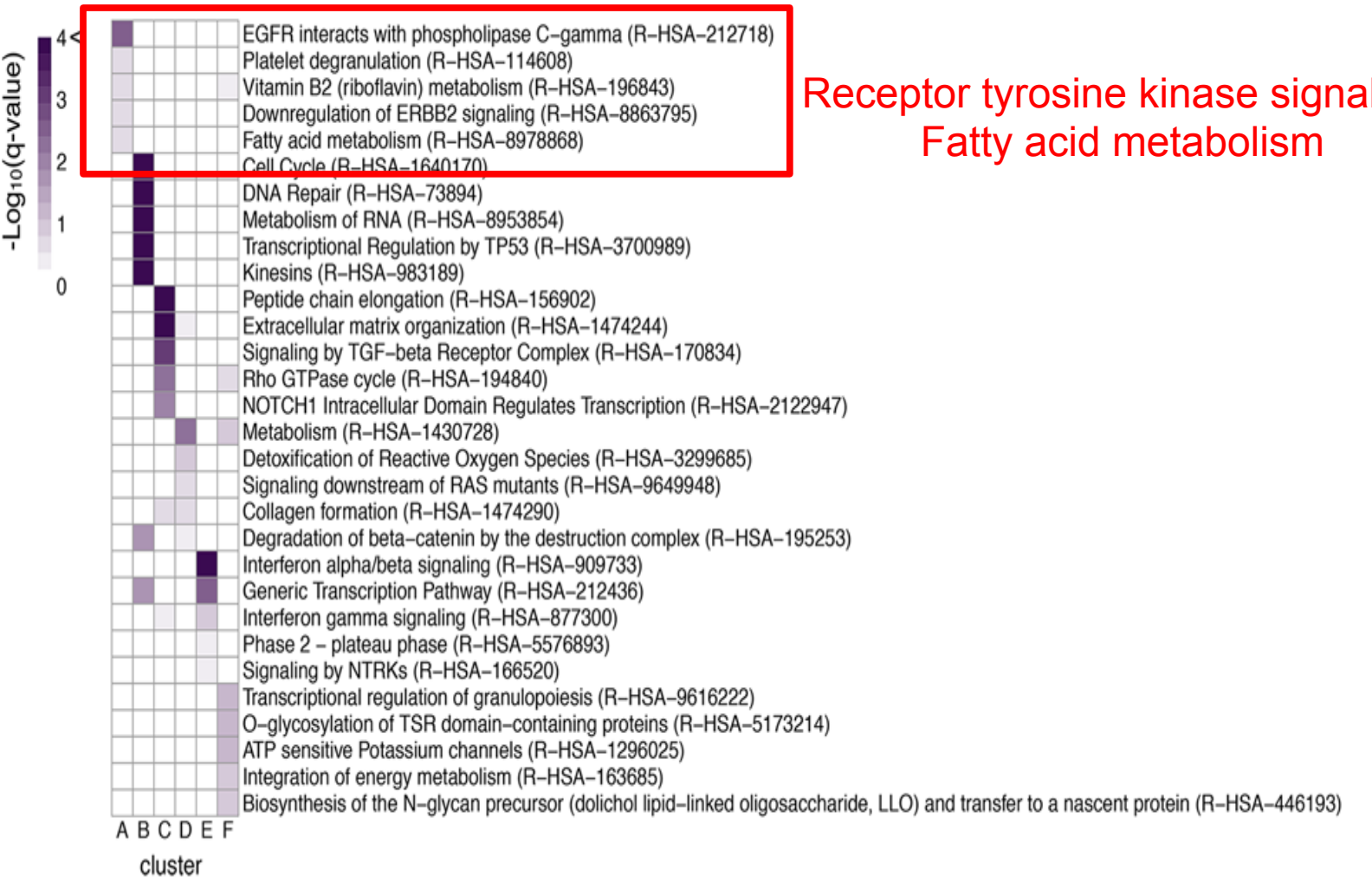


KEGG pathways database

Targetmine platform

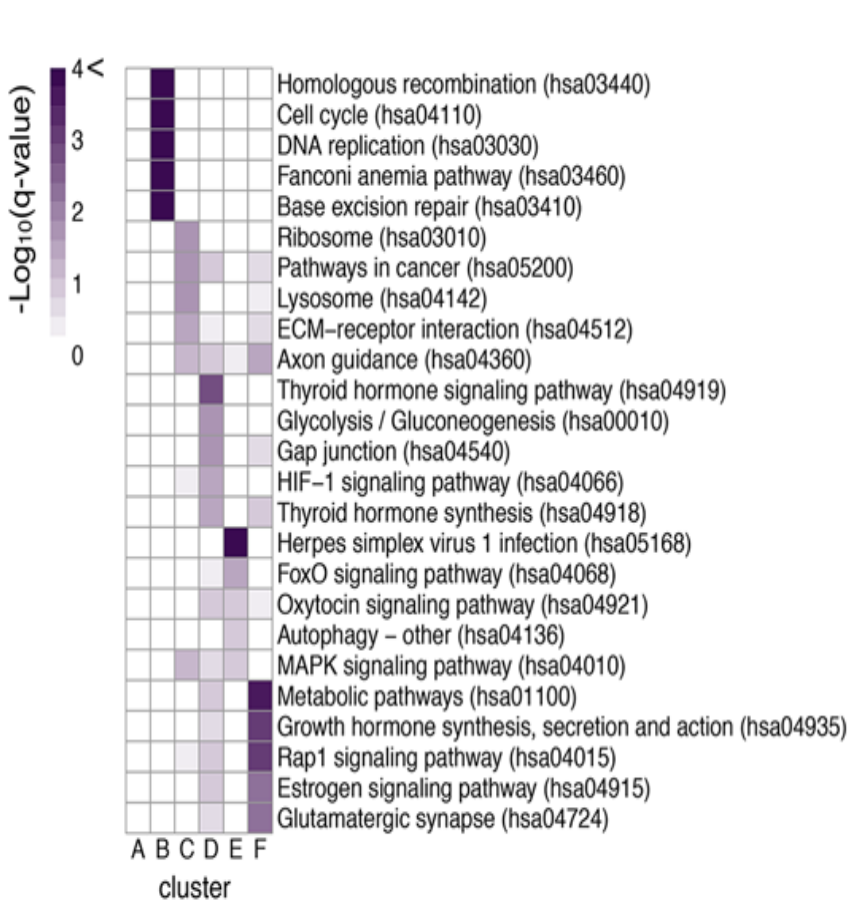


Clusters Enrichment Analysis: main points



Receptor tyrosine kinase signalling
Fatty acid metabolism

Reactome pathways database



KEGG pathways database

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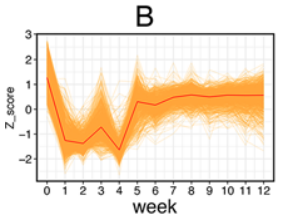
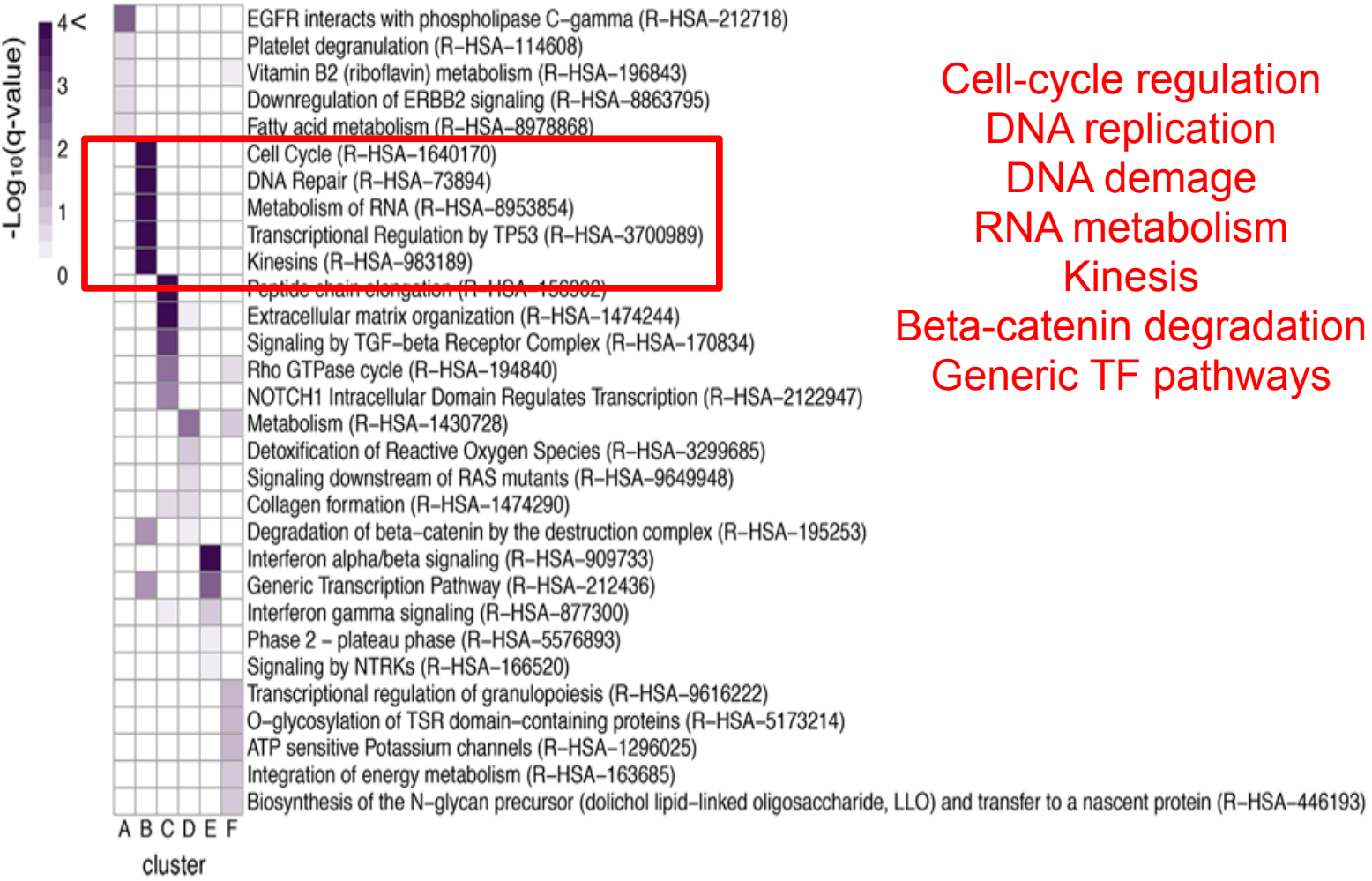
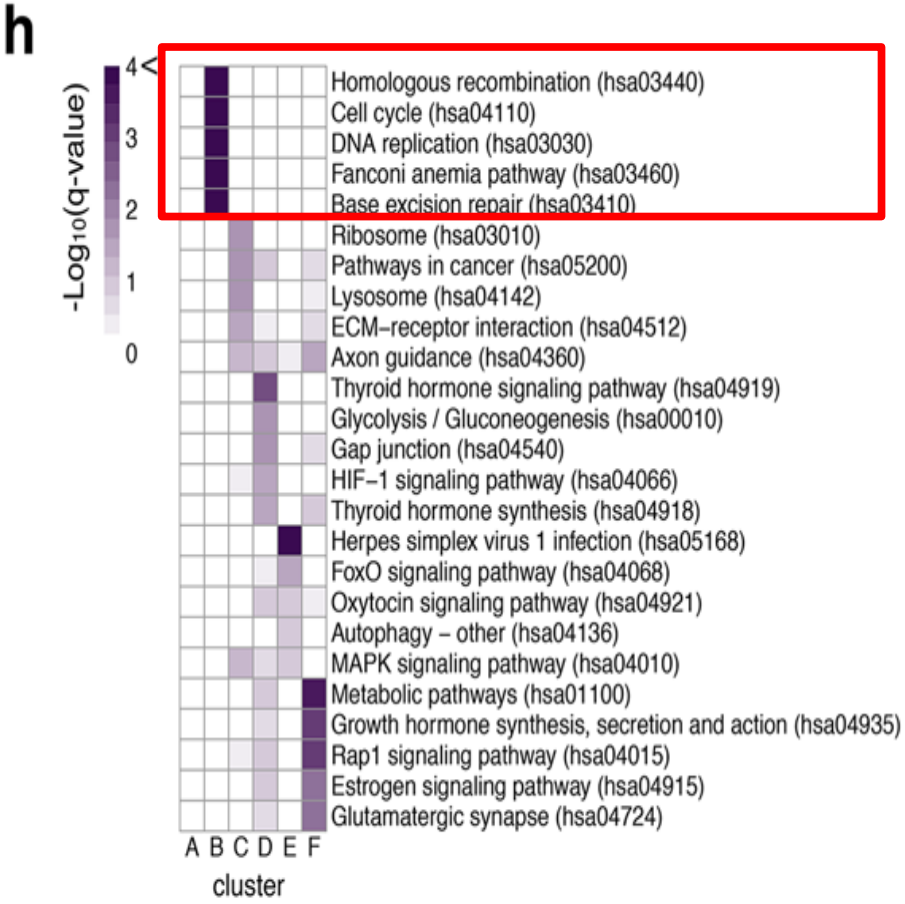




Cell-cycle regulation
DNA replication
DNA demage
RNA metabolism
Kinesis
Beta-catenin degradation
Generic TF pathways



Reactome pathways database

KEGG pathways database

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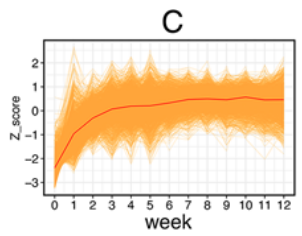
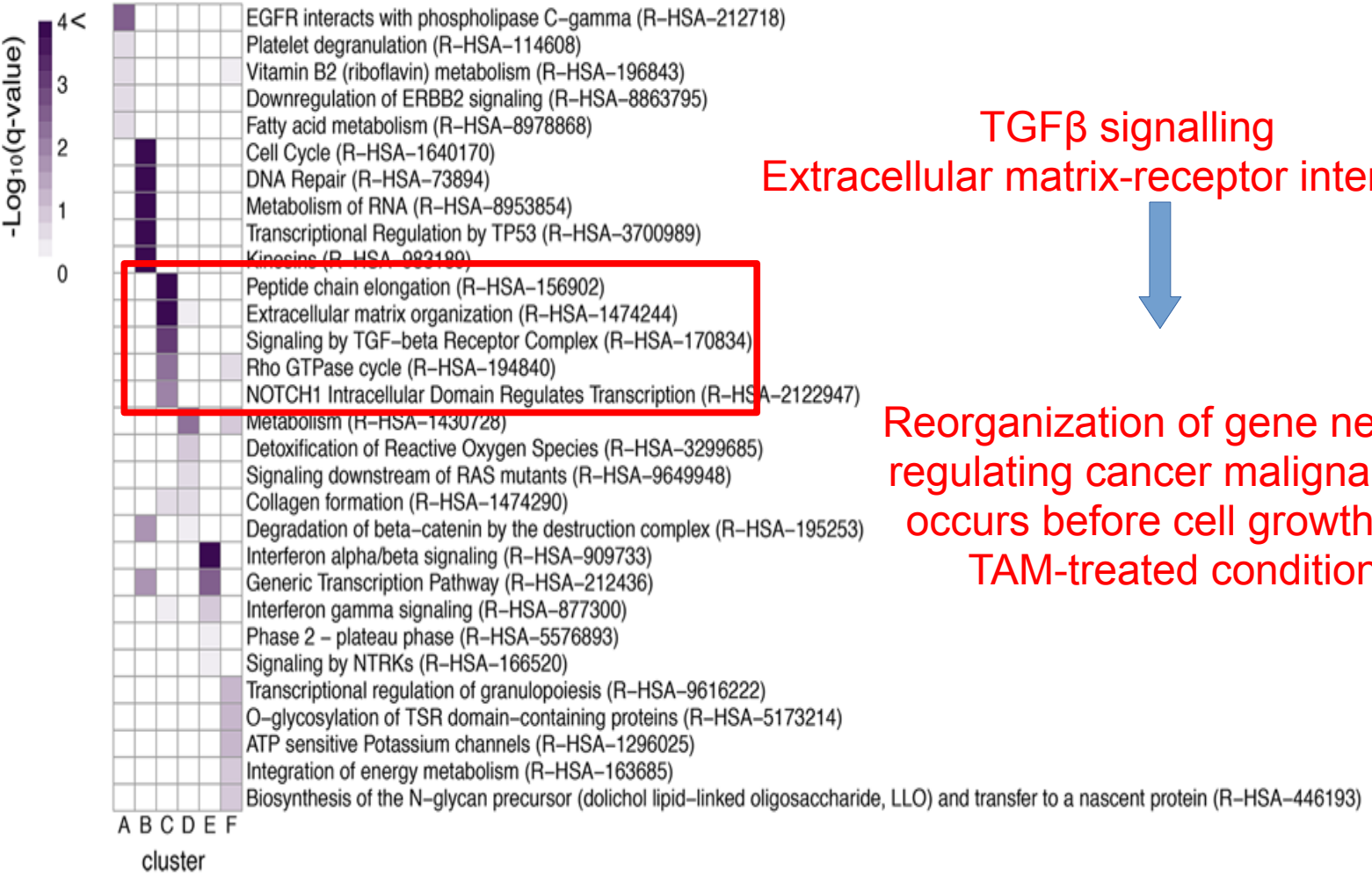
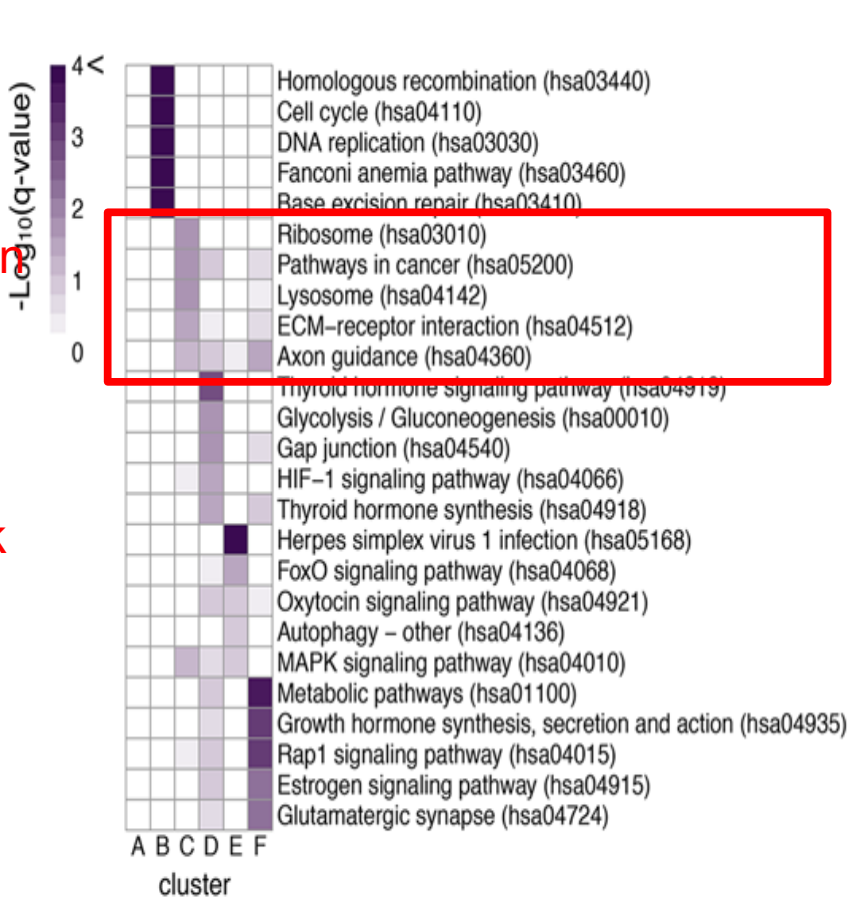




TGFβ signalling
Extracellular matrix-receptor interaction

Reorganization of gene network regulating cancer malignancy occurs before cell growth in TAM-treated condition



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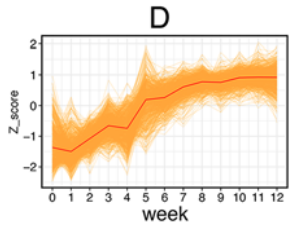
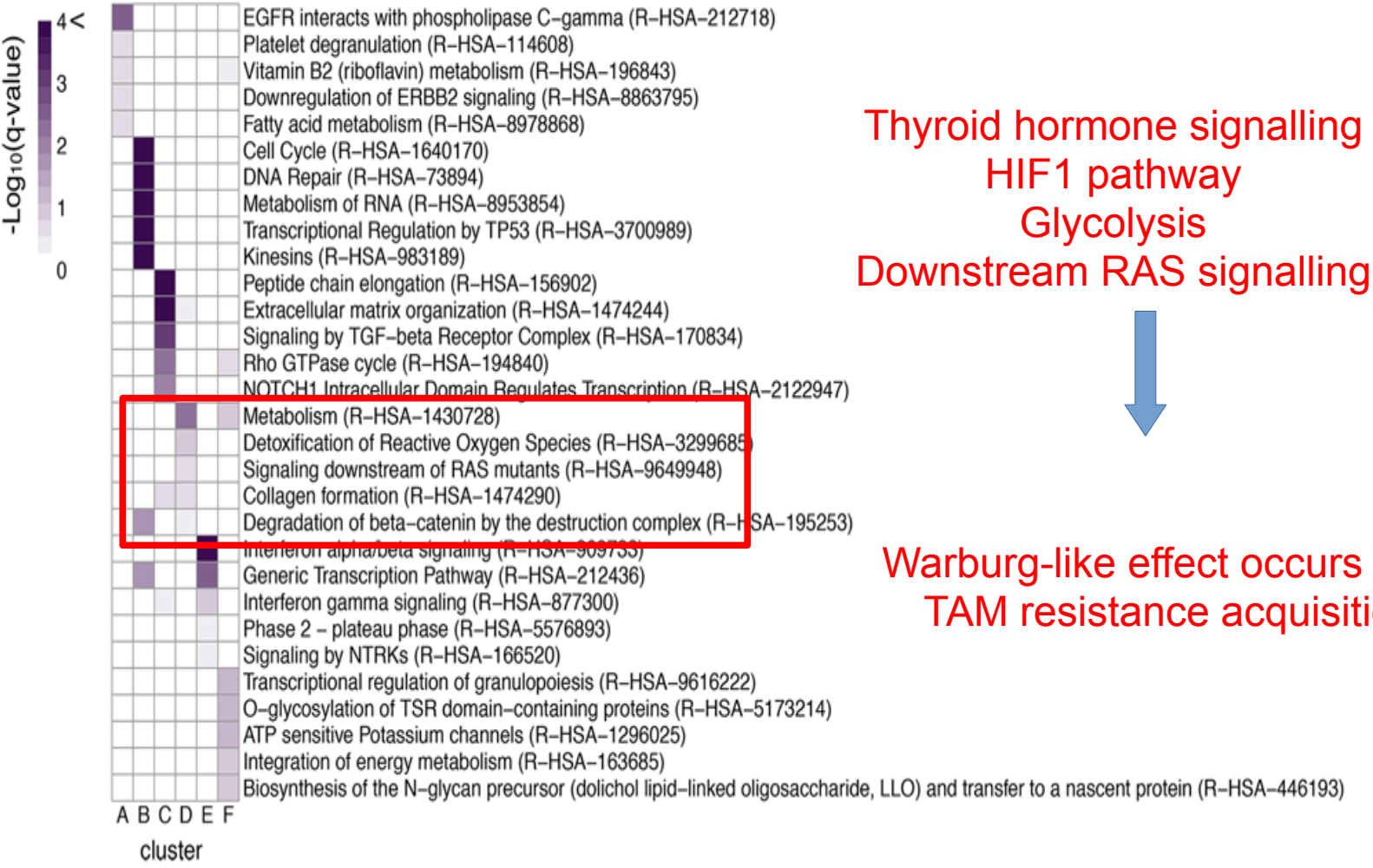
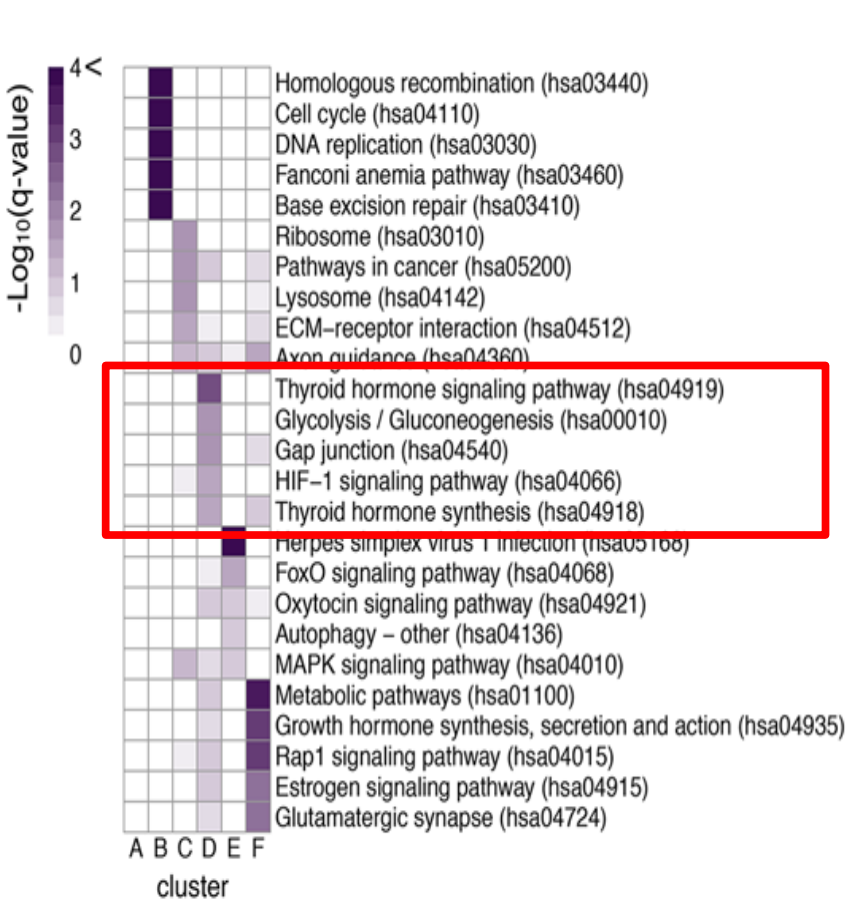




Thyroid hormone signalling
HIF1 pathway
Glycolysis
Downstream RAS signalling

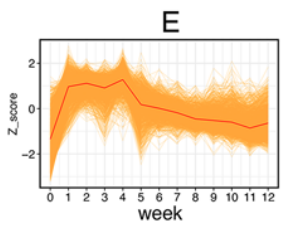
Warburg-like effect occurs with TAM resistance acquisition

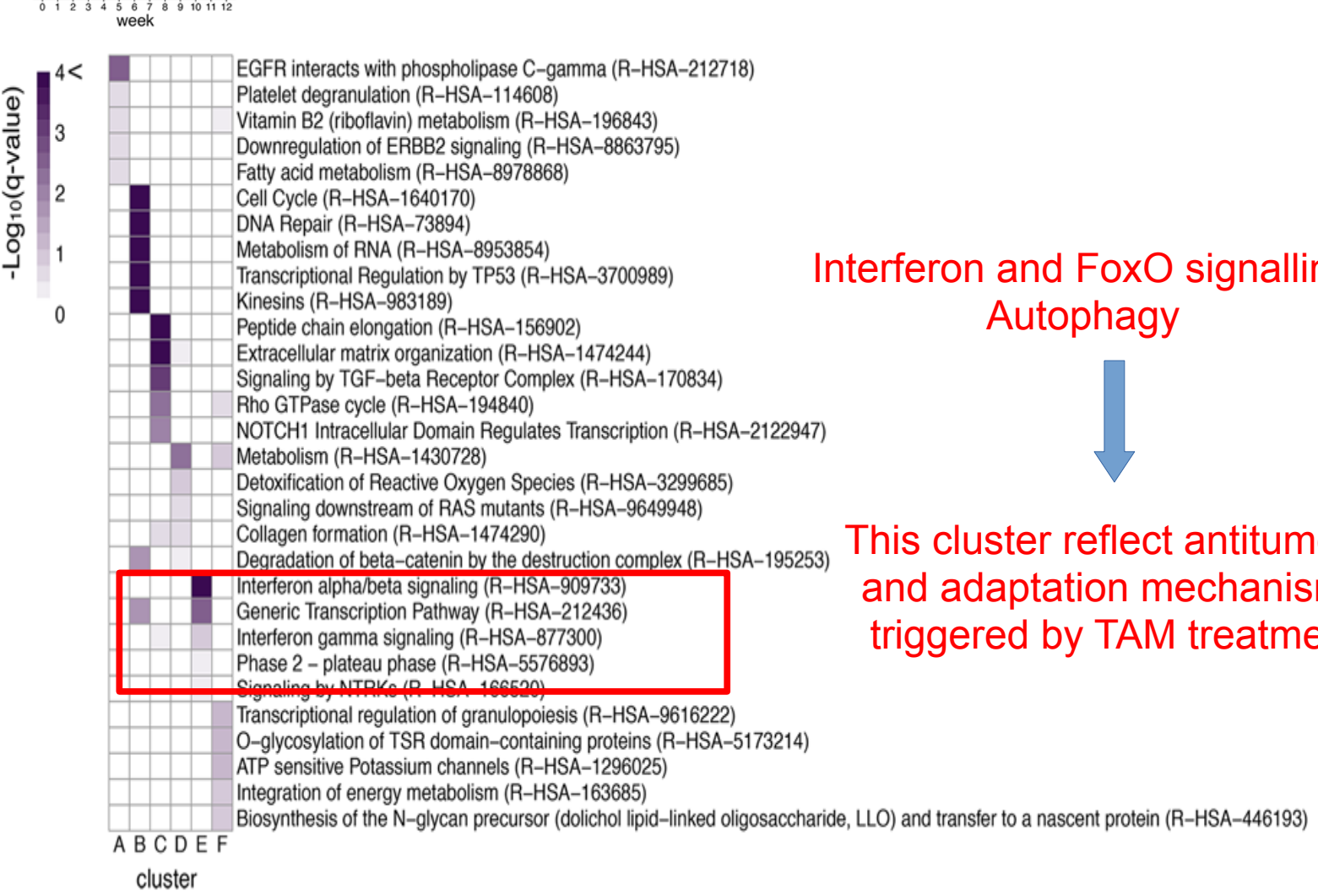


Reactome pathways database

KEGG pathways database

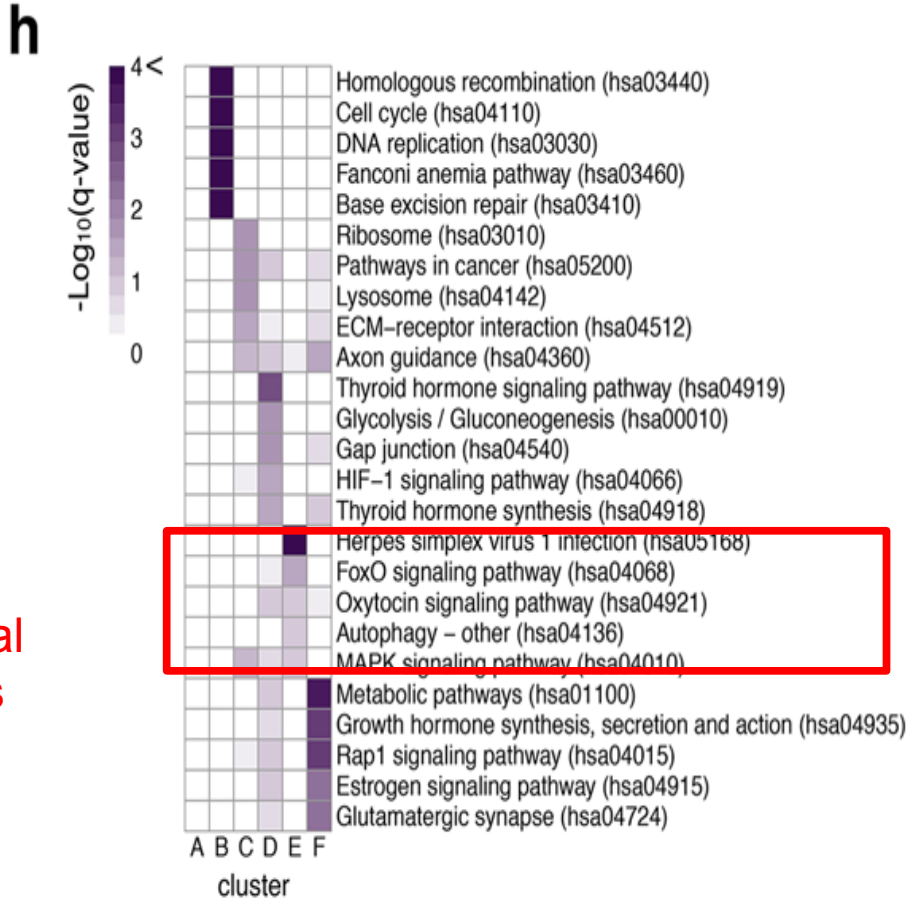
Clusters Enrichment Analysis: main points





Interferon and FoxO signalling
Autophagy

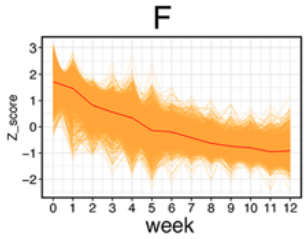
This cluster reflect antitumoral and adaptation mechanisms triggered by TAM treatment

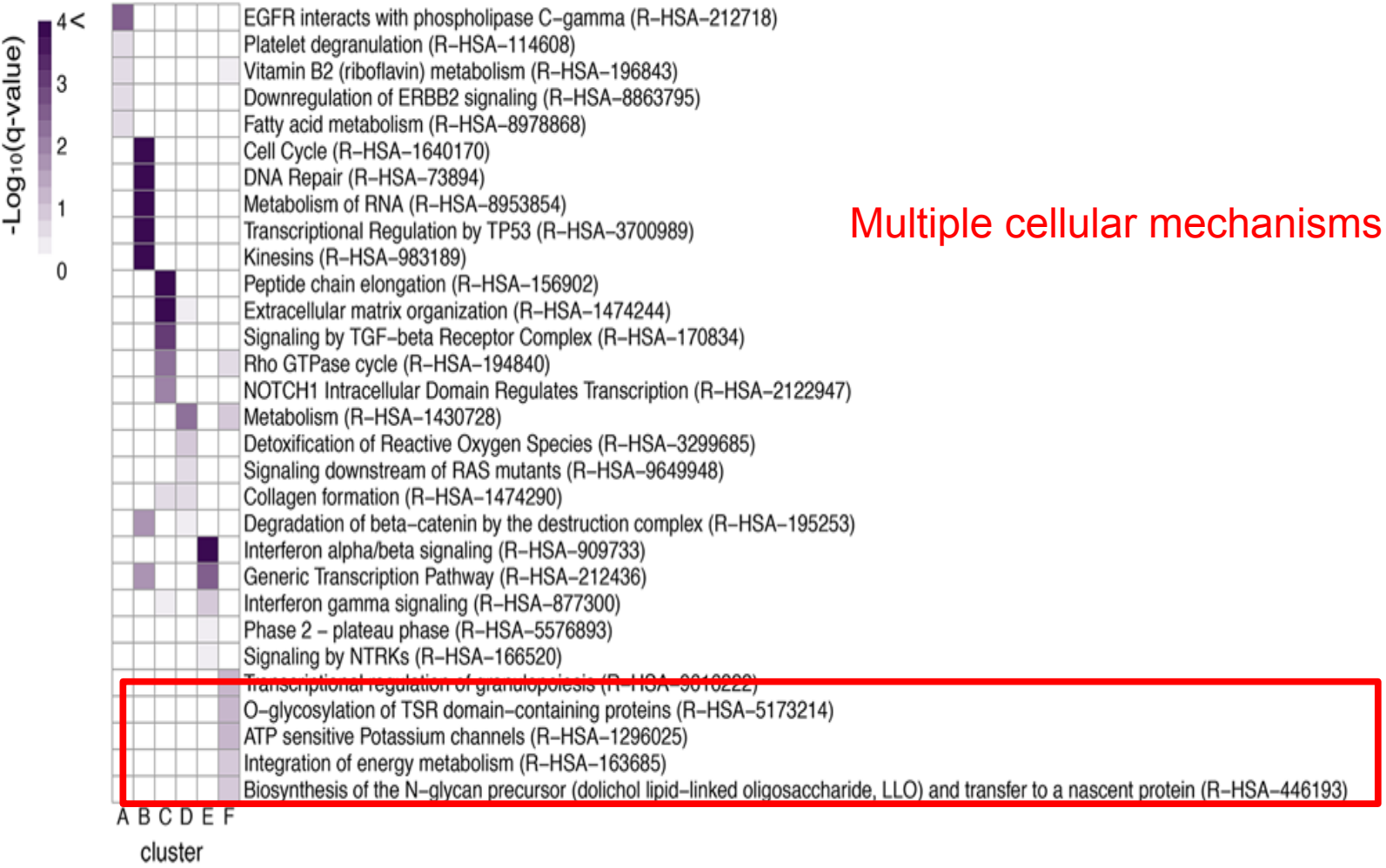


Reactome pathways database

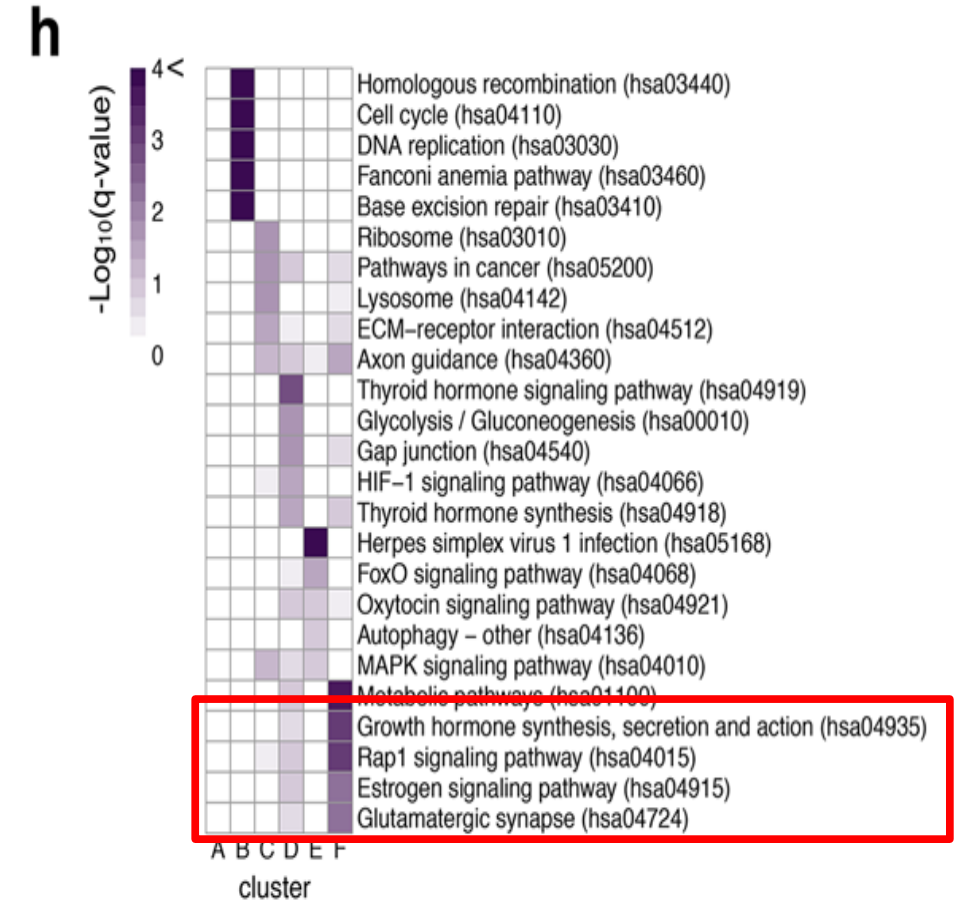
KEGG pathways database

Clusters Enrichment Analysis: main points





Multiple cellular mechanisms



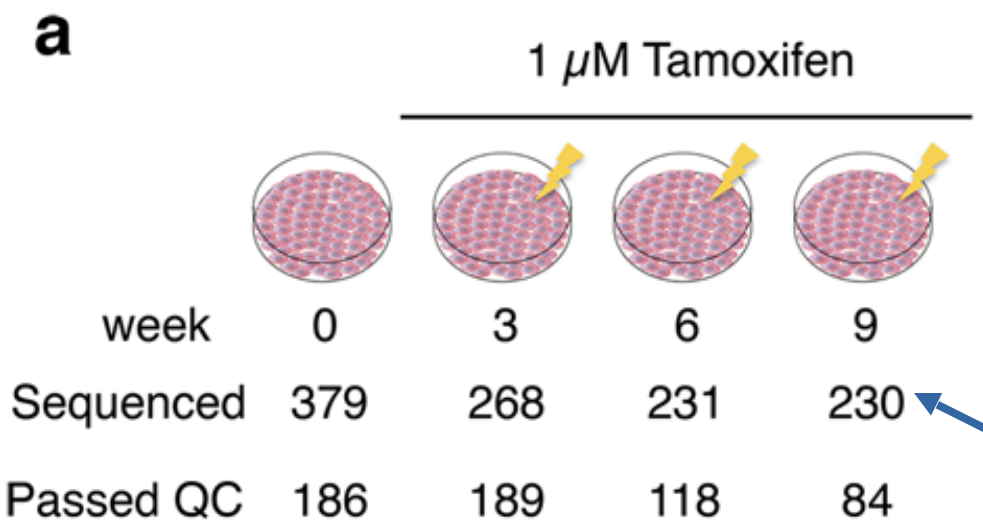
Reactome pathways database

KEGG pathways database

Single-cell RNA-seq analysis of MCF7-cells under continuous TAM treatment

Result 2

Overview of experimental procedure



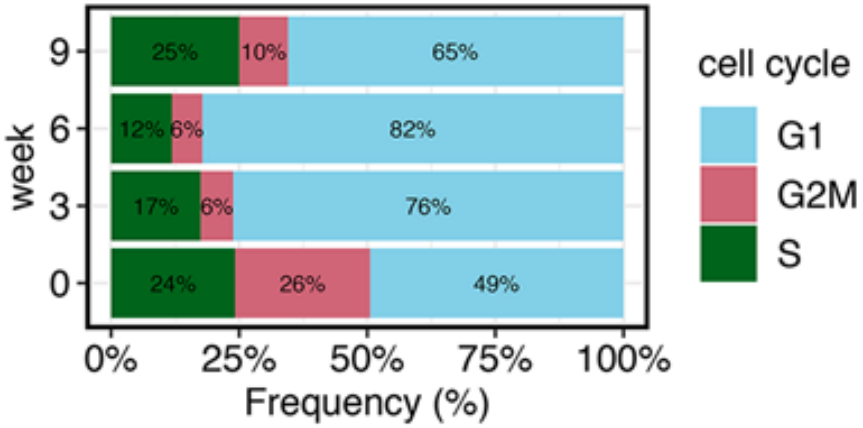
1) Four time points:

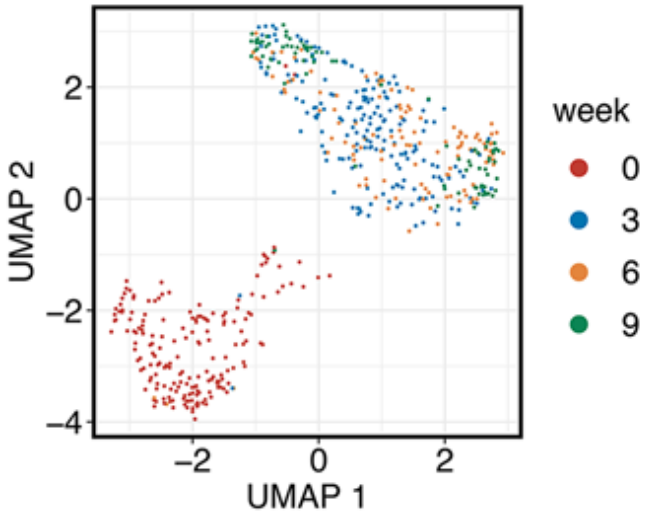
- W0: before starting TAM treatment
- W3: beginning of cell growth inhibition period
- W6: end of cell growth inhibition period
- W9: acquisition of TAM resistance

2) RNA-seq analysis of 1108 single cells

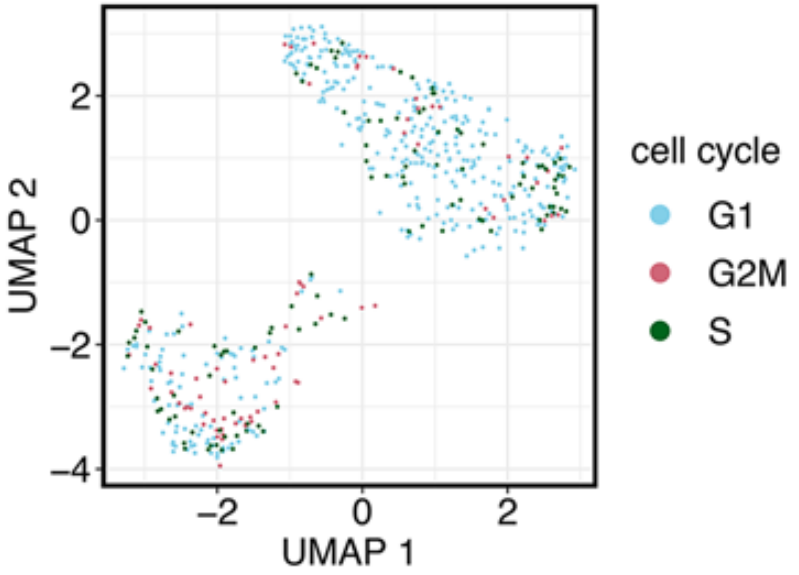
3) 577 high-quality single-cell gene expression

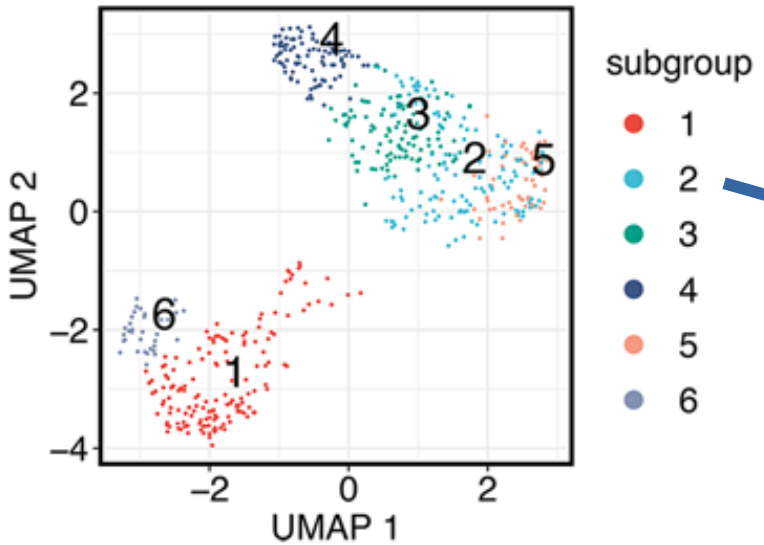
Cell-to-cell diversity: uniform manifold approximation and projection (UMAP)





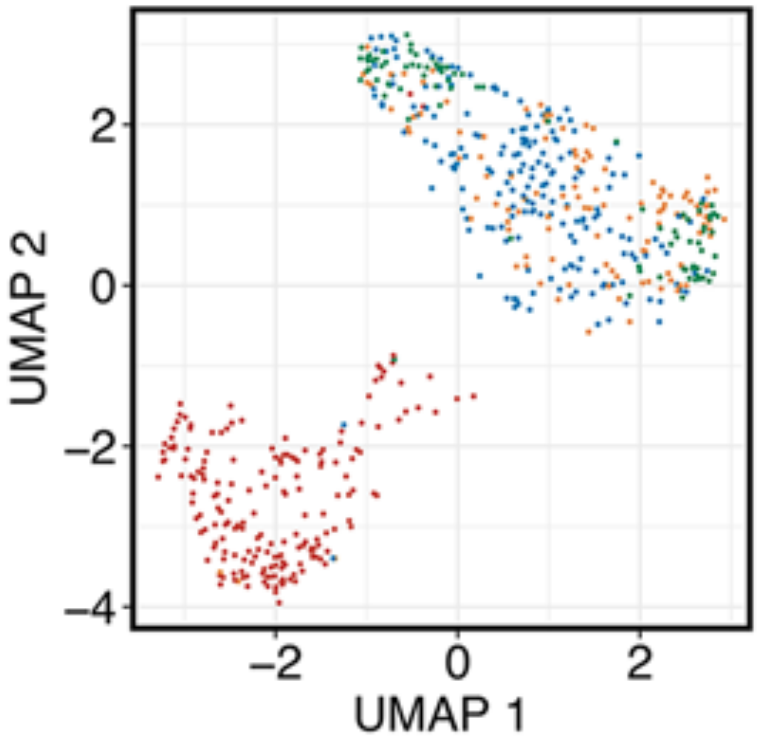
- **Two groups**

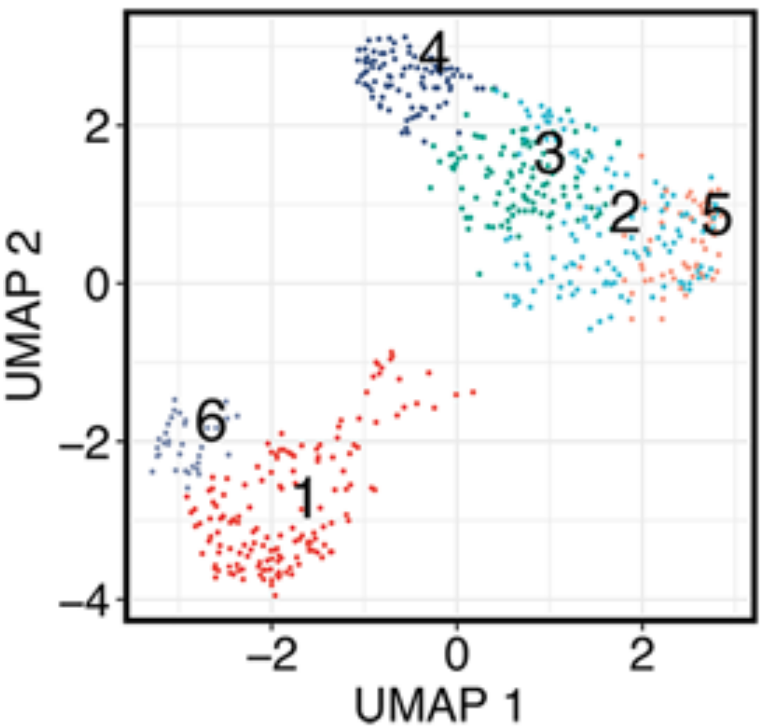


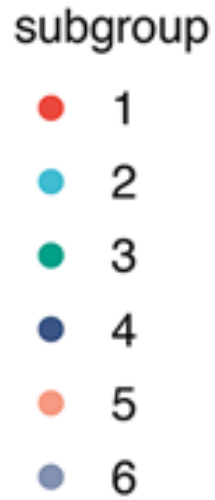


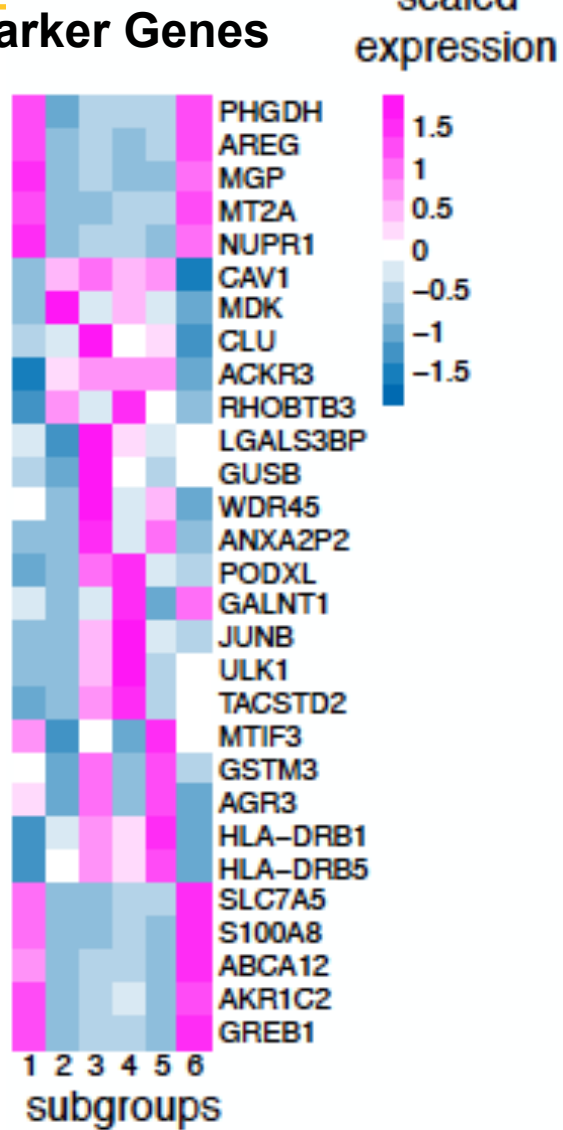
- **Six subgroups associated with weeks**

Cell-to-cell diversity: marker genes

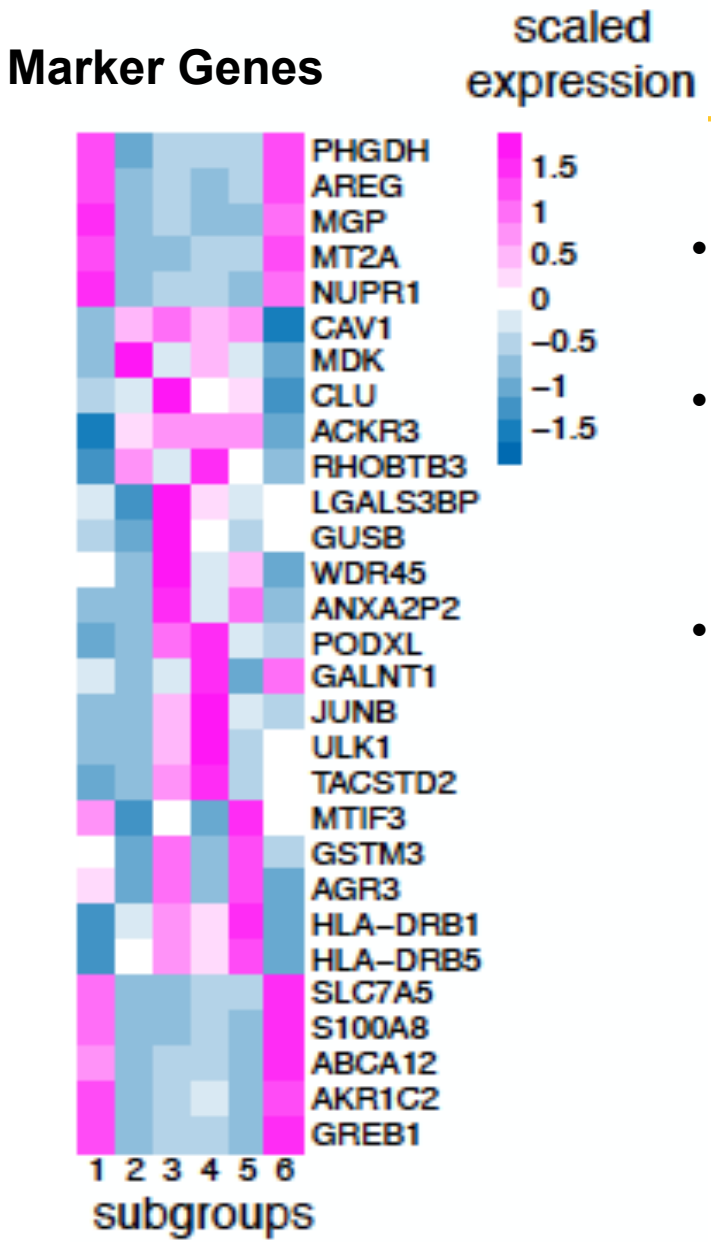




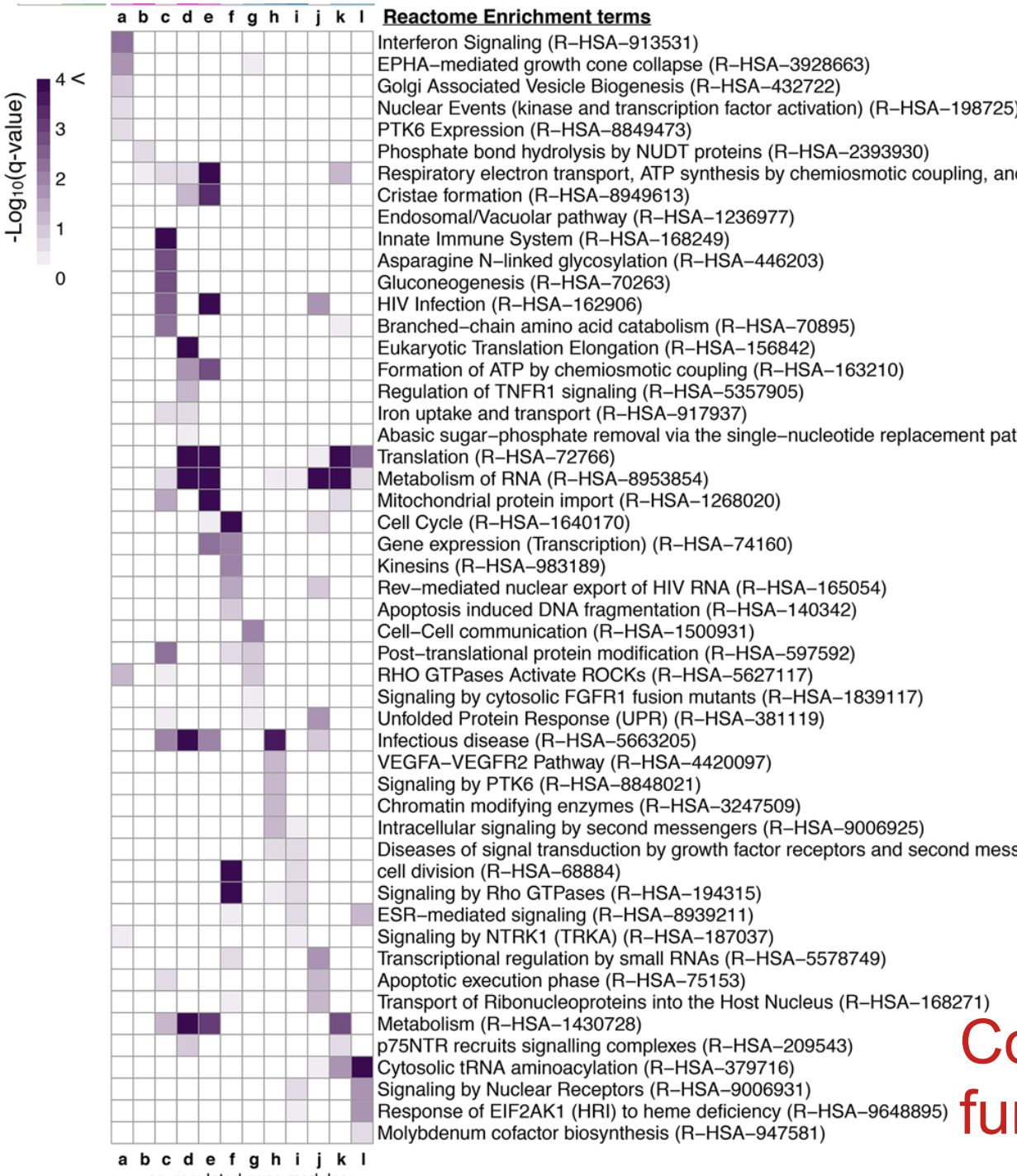


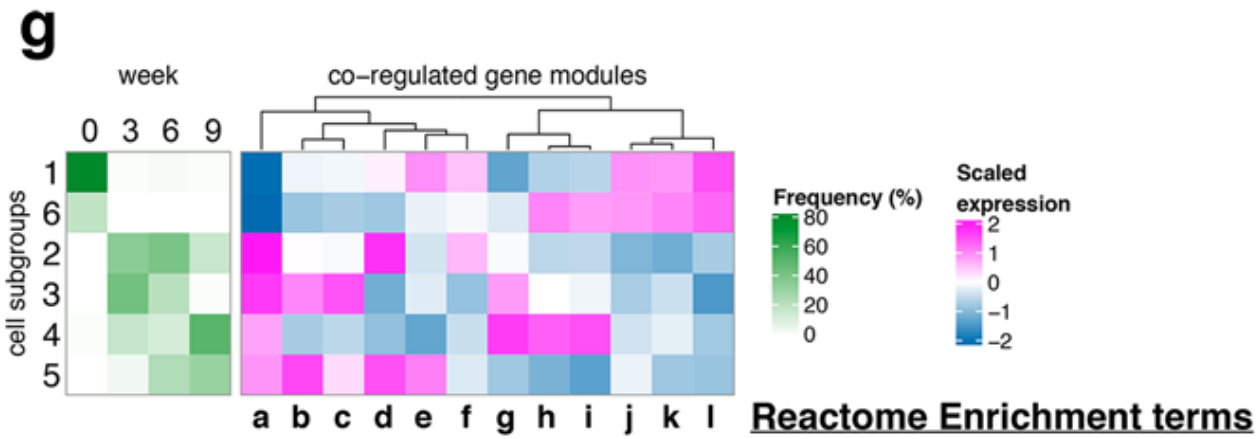


Cell-to-cell diversity: marker genes



- Sub 1 and 6: transcriptional activity of ER
- Expression level of markers in other subgroups does not clearly distinguish the cells into the subgroups
- All markers of sub 4 and 5 are also high expressed in sub 3
  - The pre-resistant sub 3 could mature into distinct resistant groups by rewiring the genetic network





Co-regulated gene modules and enriched functions

Co-regulated gene modules and enriched functions

- TAM-resistant ER-positive breast cancer cells obtained from the same parental cell line could be divided into two types

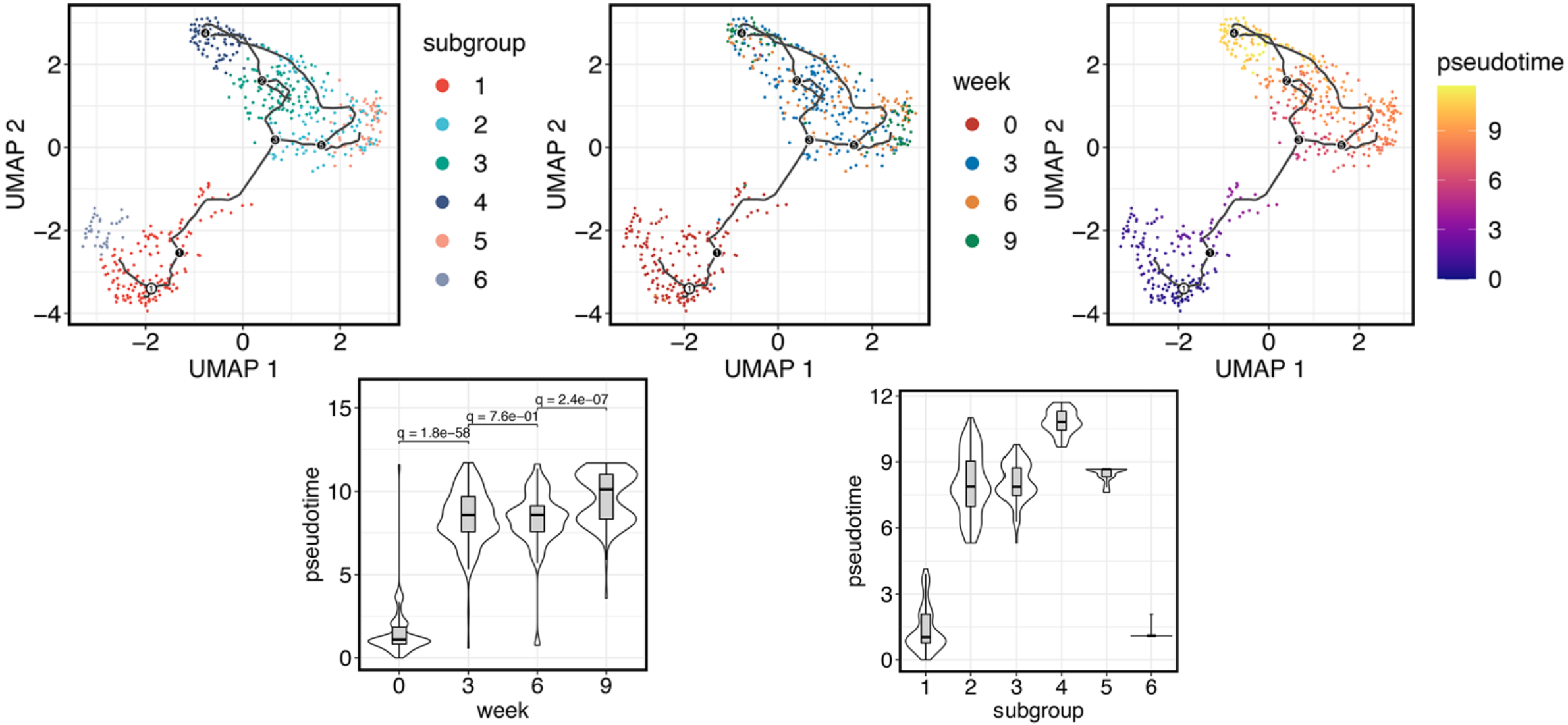
1) Sub 4: high expression level of adhesion molecules with changing epigenetic status

1)Sub 5: rewiring of metabolic network

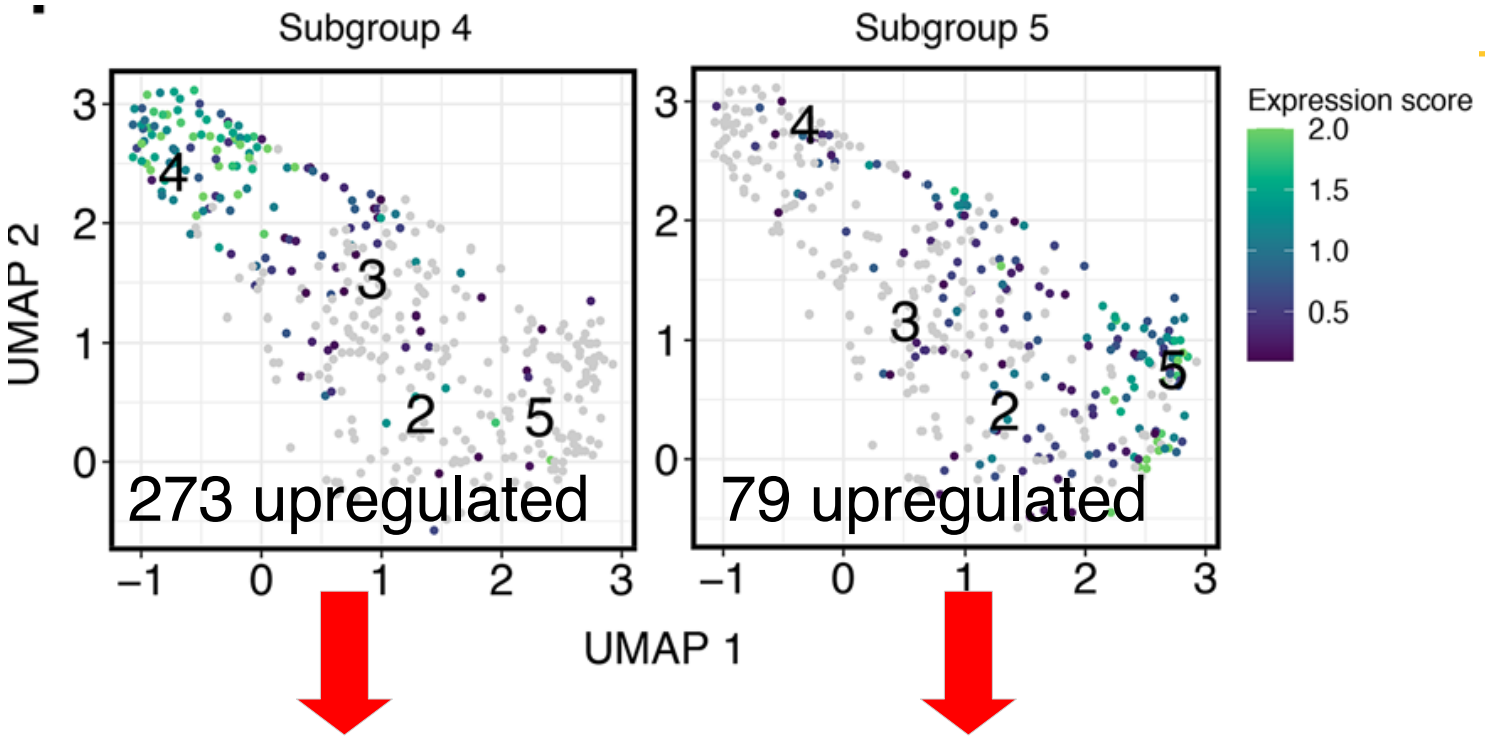
Trajectory analysis of TAM resistance

Result 3

PSEUDOTIME ANALYSIS: CONFIRMING CELL TRANSITION TRAJECTORY INTO TWO DIFFERENT TYPE OF RESISTANT SUBGROUPS

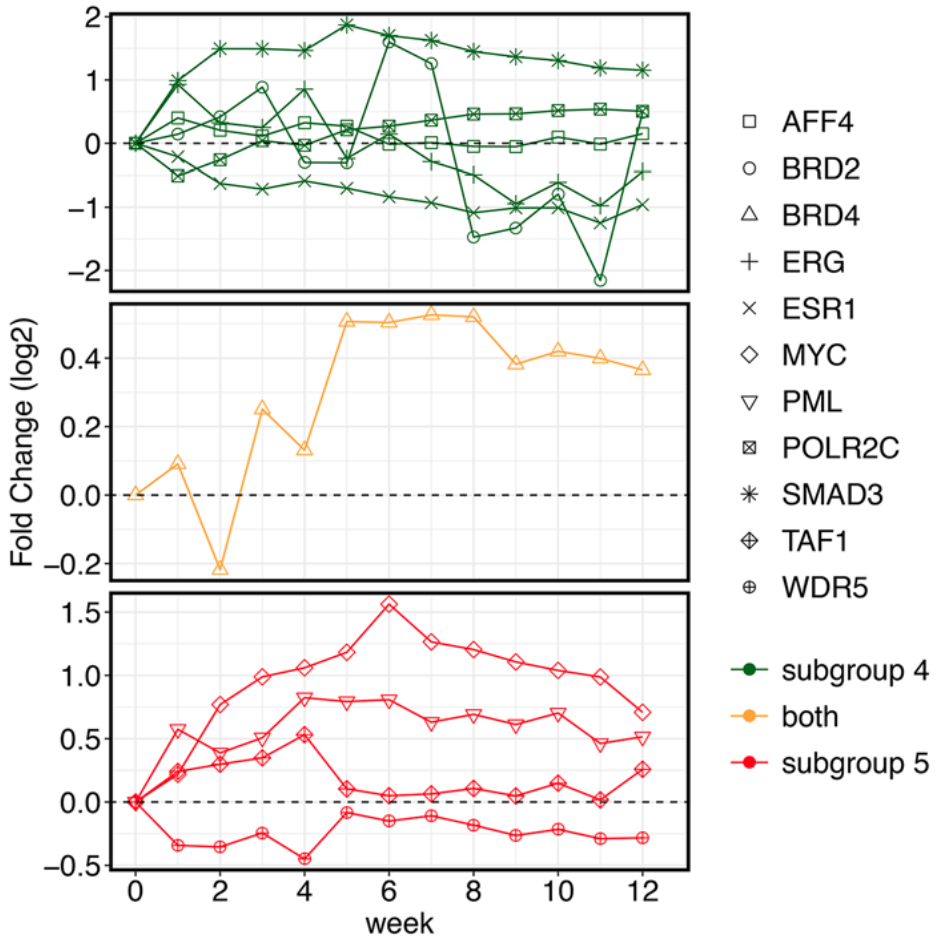


Identifying important players in the emergence of subgroups 4 and 5



ID	TFs	Cell	Log10 q-value	Fold enrichment
SRX5089555	BRD4	MCF-7	-38.7	4.4
SRX7692443	POLR2C	MCF-7	-34.3	2.0
SRX2897220	ESR1	Breast cancer cells	-29.0	1.7
SRX4377547	BRD2	HCC1806	-26.9	1.6
SRX7615734	AFF4	MCF-7	-26.3	6.6
SRX4377545	BRD2	SUM 159PT	-25.8	1.7
SRX4377548	BRD2	HCC1806	-25.0	1.6
SRX5757564	SMAD3	SUM 159PT	-24.9	1.6
SRX4377540	BRD2	MDA-MB-231	-24.7	1.6
SRX063918	ERG	MCF-7	-21.7	1.6

ID	TFs	Cell	Log10 q-value	Fold enrichment
SRX3958390	WDR5	Breast cancer cells	-38.1	80.6
SRX795223	BRD4	SUM 159PT	-33.4	13.0
SRX2585780	MYC	MDA-MB-231	-26.4	8.8
SRX2944452	MYC	MCF 10A	-26.2	9.9
SRX1758507	NELFE	MCF-7	-24.9	6.3
SRX190267	TAF1	MCF-7	-20.7	3.7
SRX190294	PML	MCF-7	-19.6	3.5
SRX103003	MYC	MCF-7	-18.0	3.3
SRX1131555	BRD4	SUM 1315M02	-17.7	6.3
SRX1131571	BRD4	T-47D	-17.3	3.9



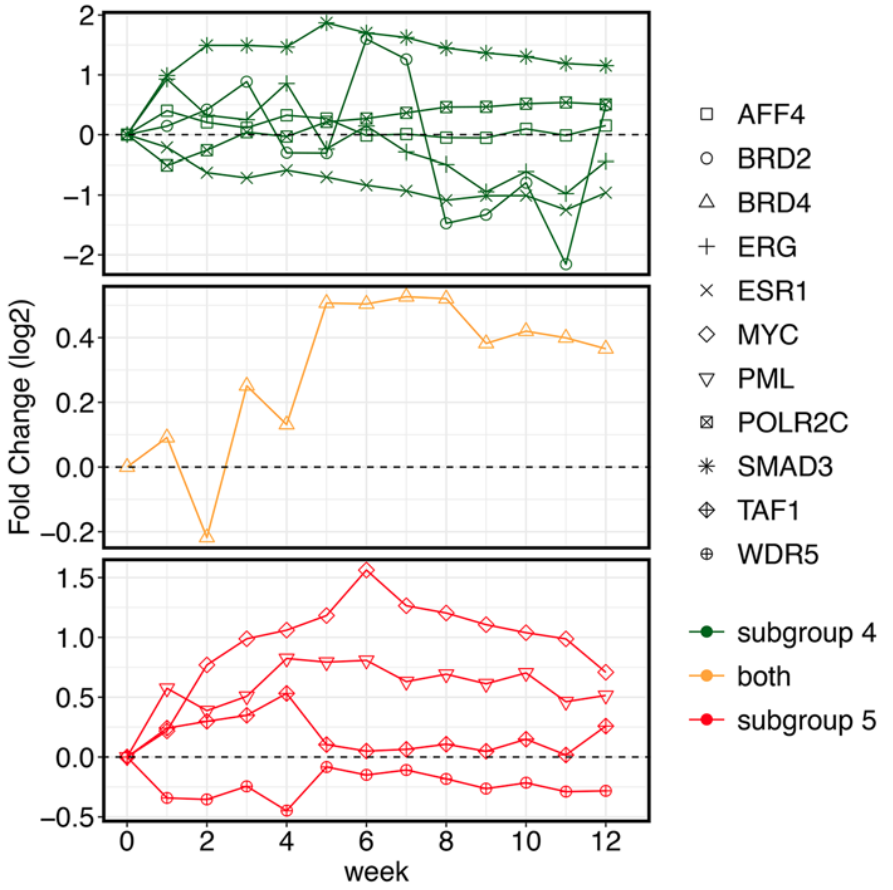
Identifying important players in the emergence of subgroups 4 and 5

- Tfs BRD4 and BRD2 control the up-regulated genes in subgroup 4
  - These recognize acetylated histones and act as super enanchers

- Oncogenic Tfs SMAD3 and ERG

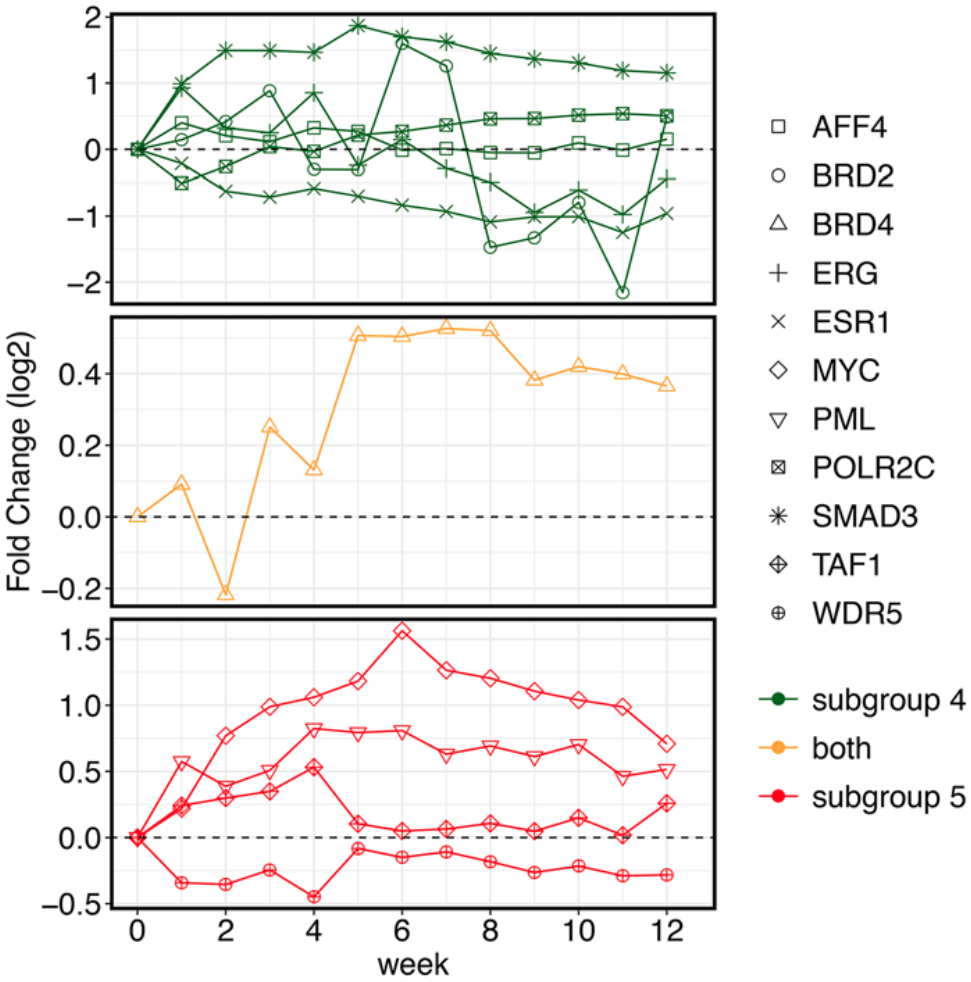
- Cells in subgroups 4 have different status due to
- epigentic alterations

- Tfs MYC, TAF1, PML, and BRD4 control the
- up-regulated genes in subgroup 5



Identifying important players in the emergence of subgroup 5

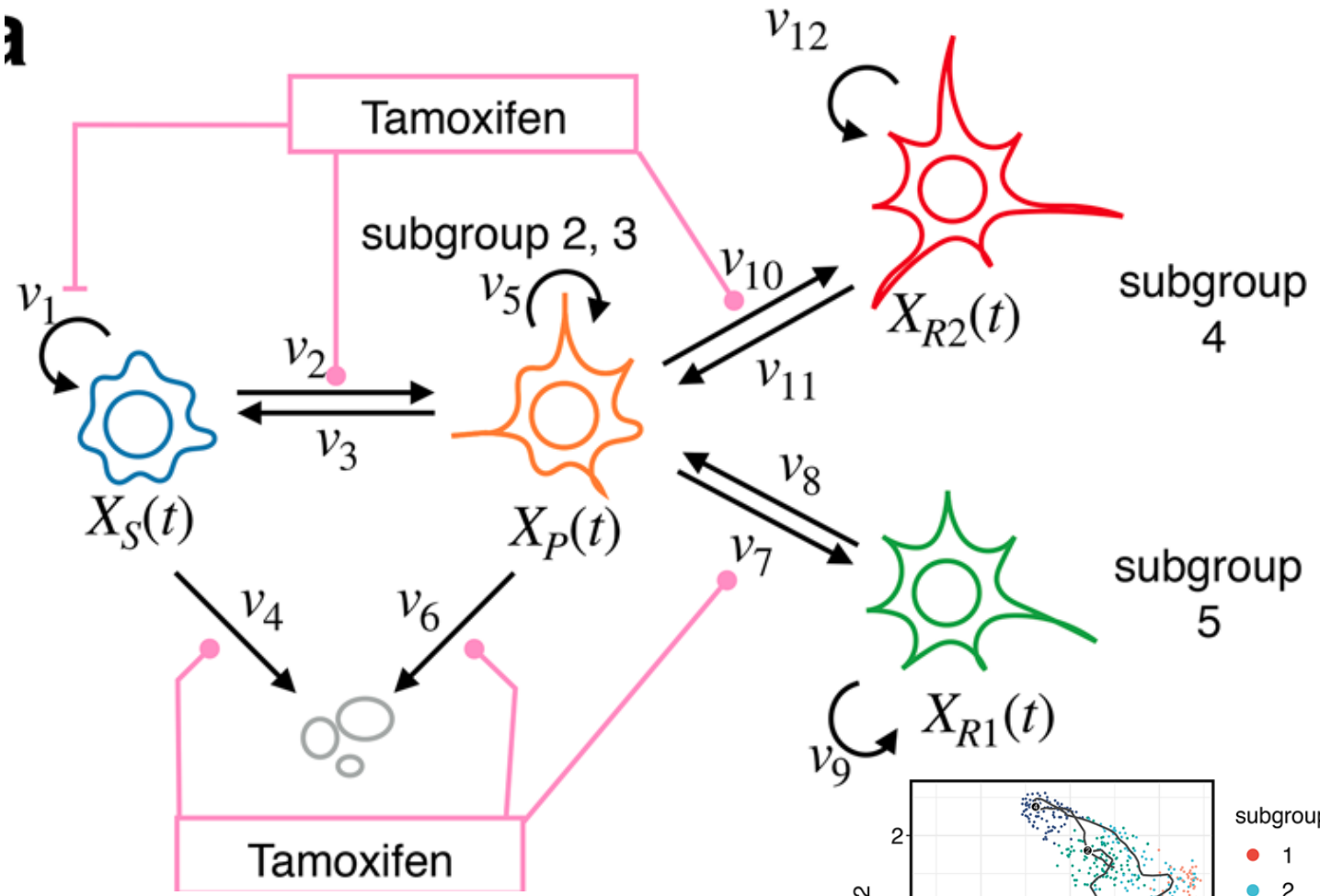
- Tfs WDR5 might regulate genes in subgroup 5
- Oncogenic Tfs SMAD3 and ERG
- Cells in subgroups 4 have different status due to
- epigentic alterations

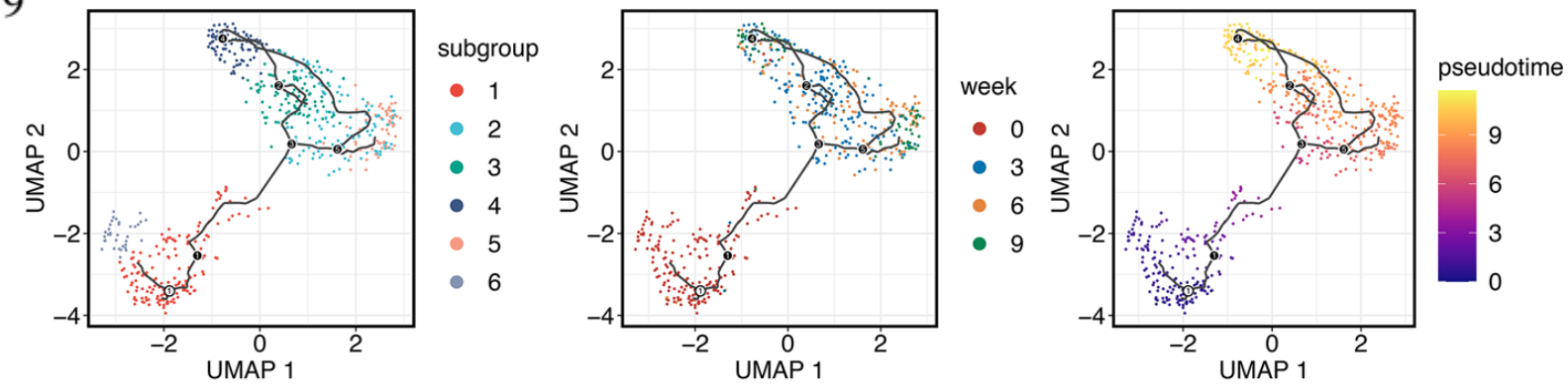


Mathematical modeling of TAM resistance acquisition process

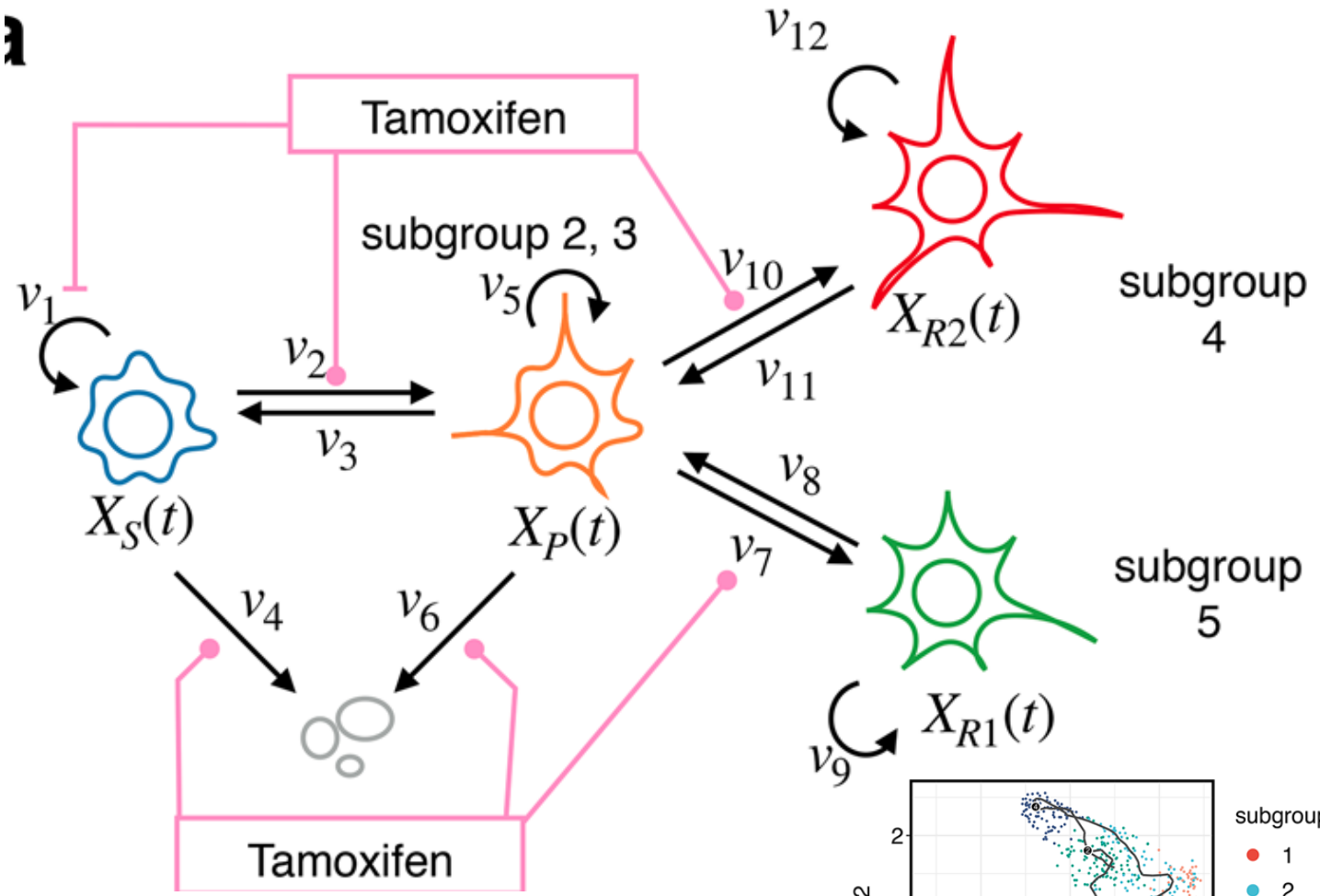
Result 4

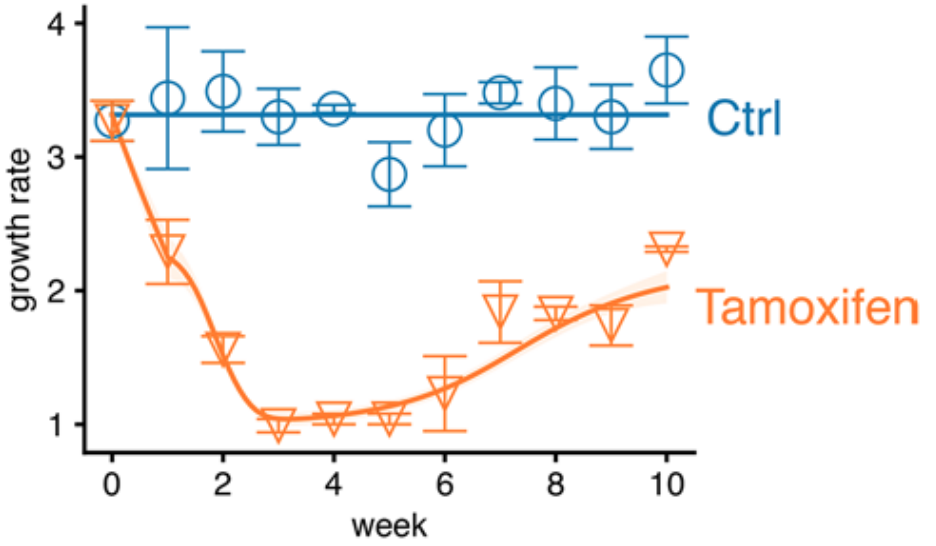
ODE model of TAM resistance acquisition process

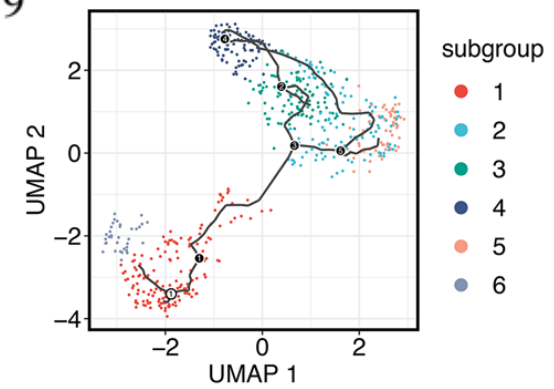


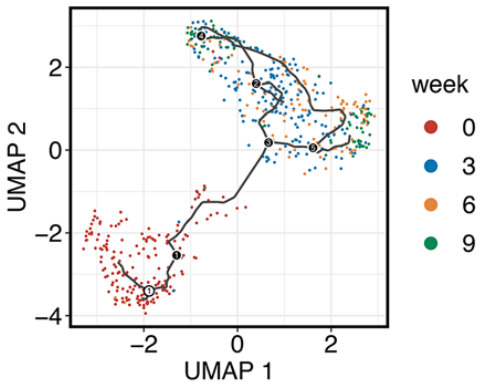


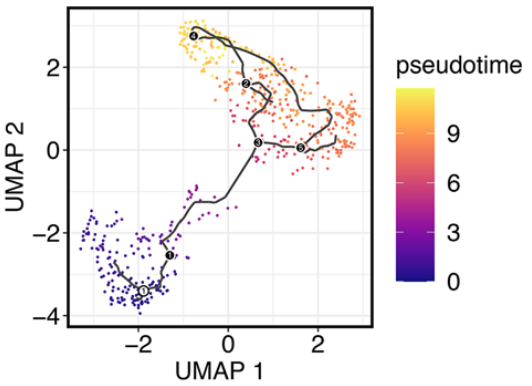
ODE model of TAM resistance acquisition process



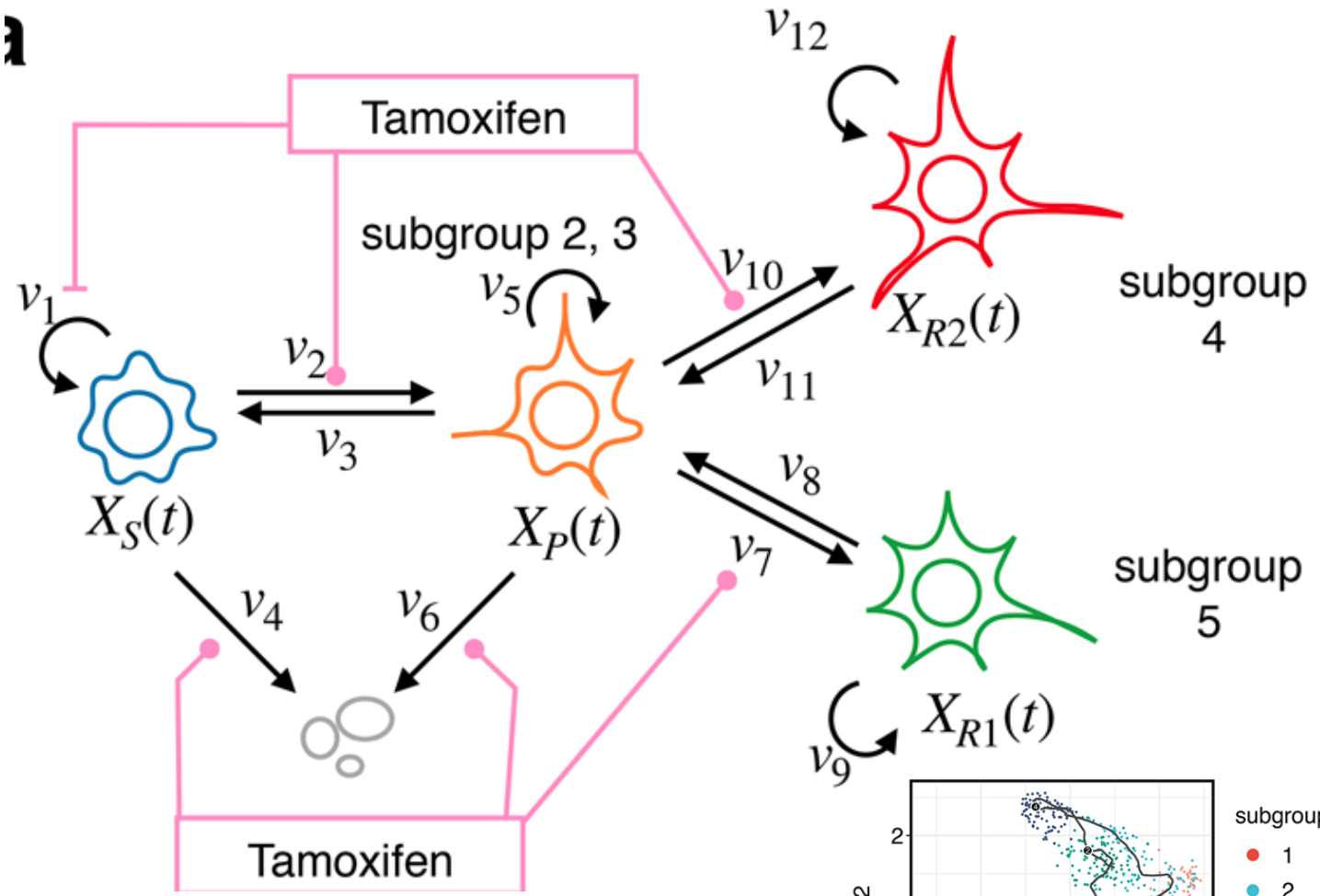


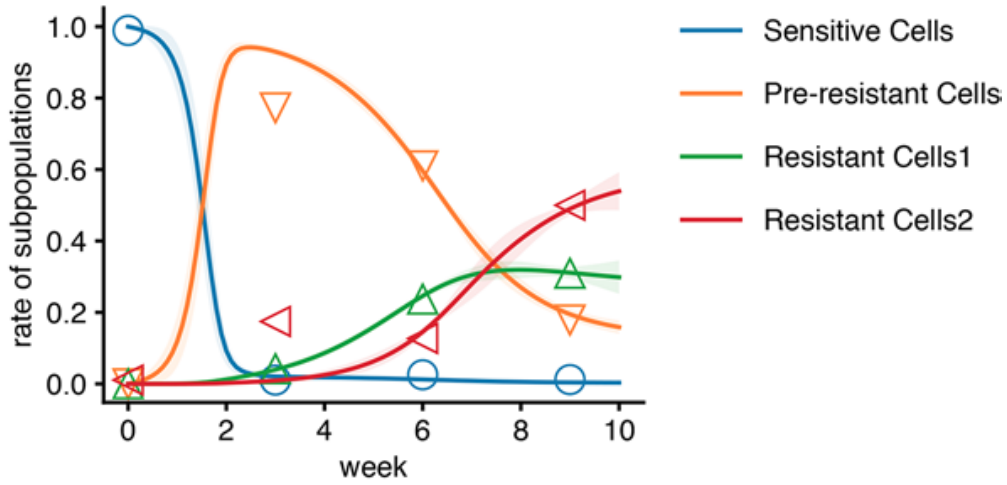


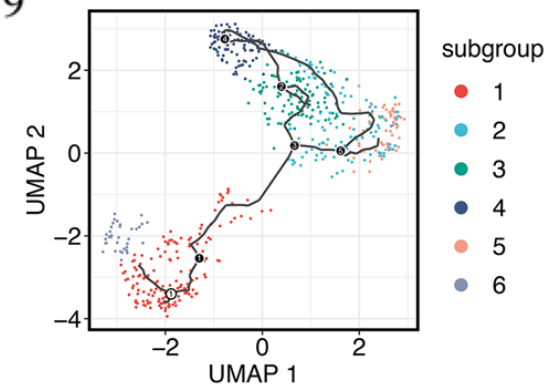


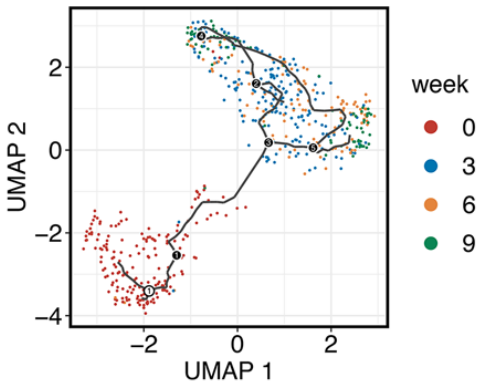


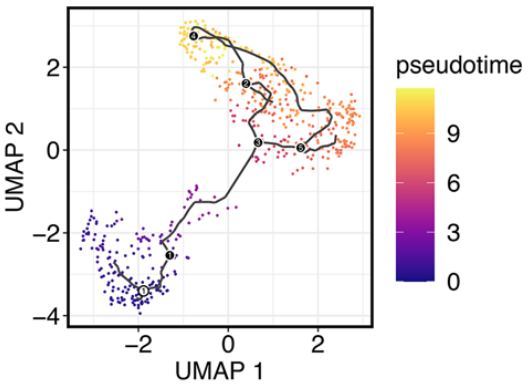
ODE model of TAM resistance acquisition process

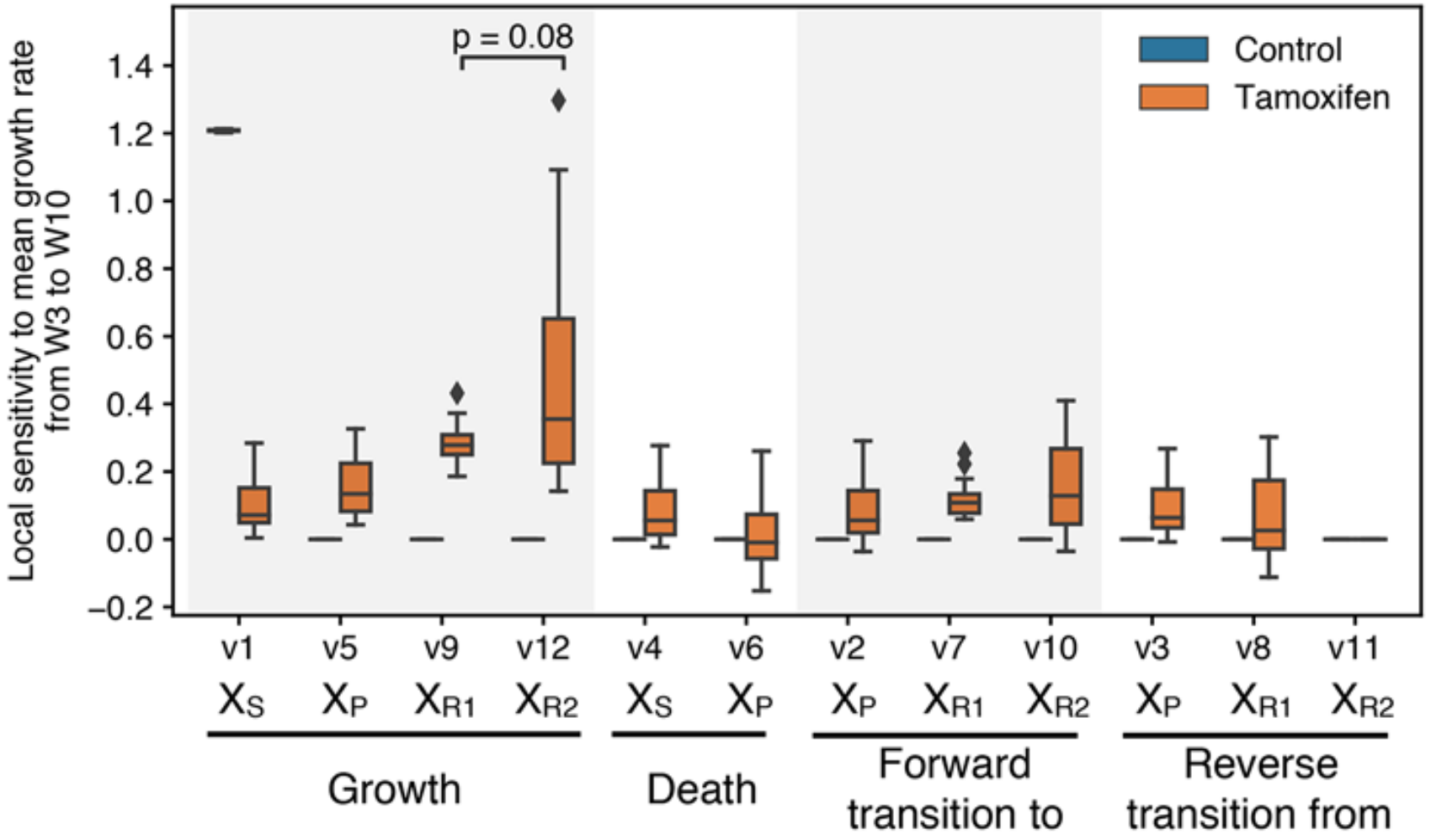


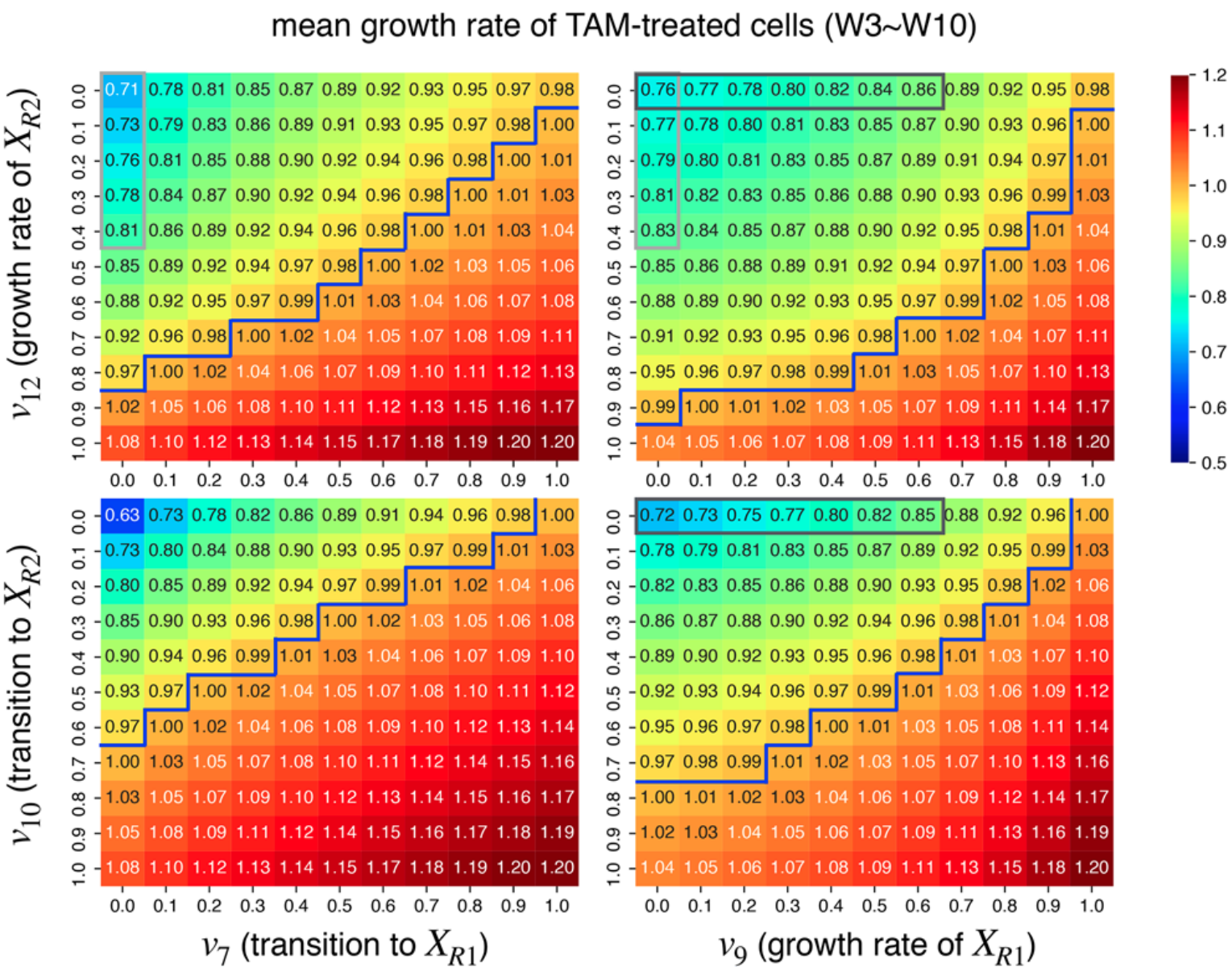








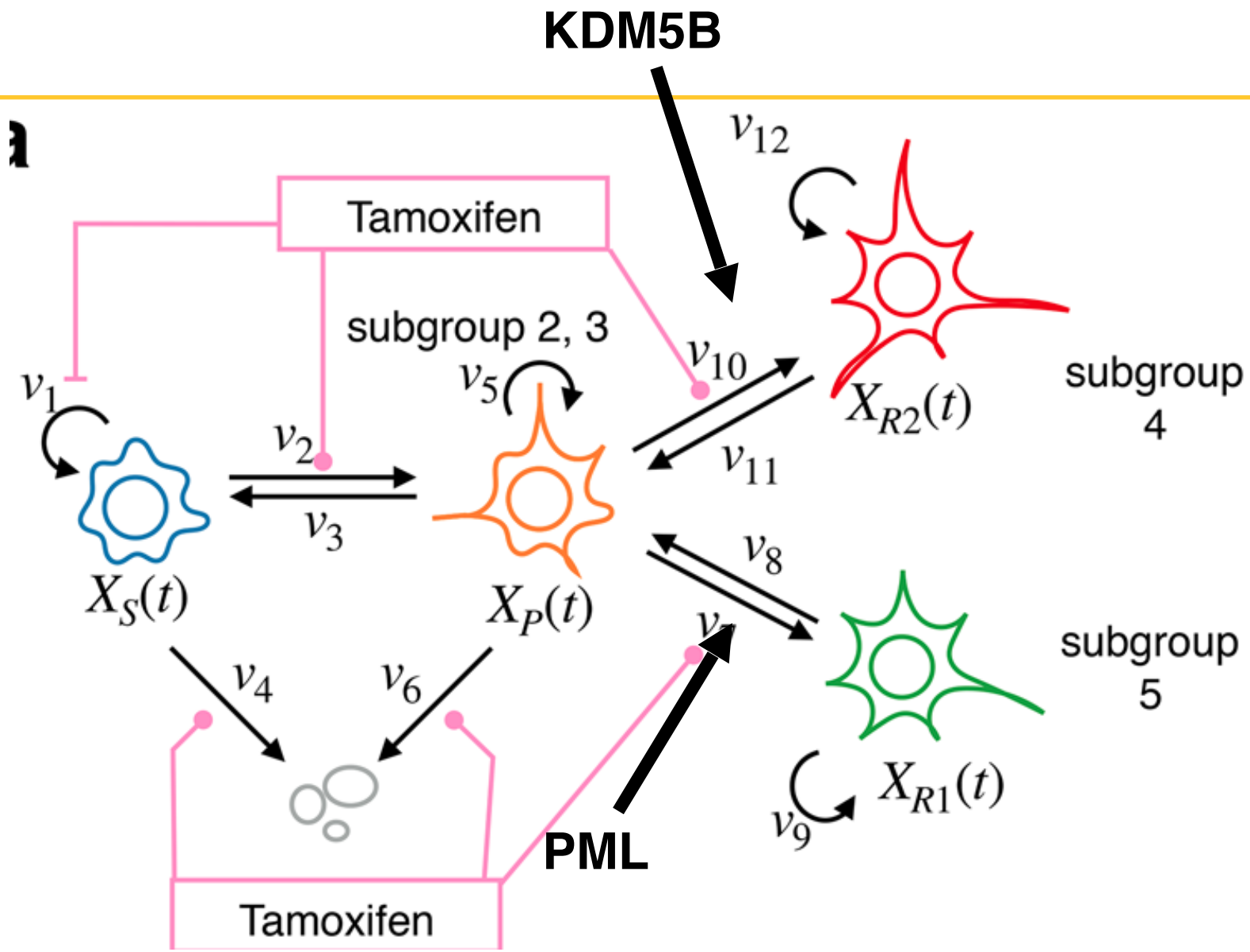

p = 0.08
Control
Tamoxifen
Local sensitivity to mean growth rate from W3 to W10
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
−0.2
v1
v5
v9
v12
v4
v6
v2
v7
v10
v3
v8
v11
X_S
X_P
X_{R1}
X_{R2}
X_S
X_P
X_P
X_{R1}
X_{R2}
X_P
X_{R1}
X_{R2}
Growth
Death
Forward transition to
Reverse transition from


mean growth rate of TAM-treated cells (W3~W10)
v_{12} (growth rate of X_{R2})
v_{10} (transition to X_{R2})
v_7 (transition to X_{R1})
v_9 (growth rate of X_{R1})
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0
1.2 1.1 1.0 0.9 0.8 0.7 0.6 0.5

Inhibition of molecules mediating the generation of two resistant subpopulations induces regression in the pre-resistant stage

Result 5

Regression before complete acquisition of TAM resistance



Regression before complete acquisition of TAM resistance: valiation