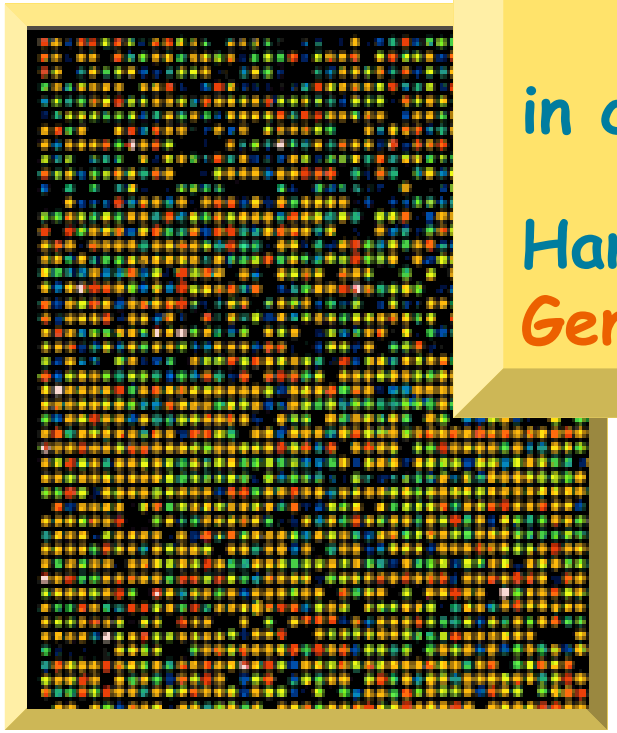
# Expression Profiling in Drug Discovery



Jutta Heim, Arndt Brachat
Novartis Pharma Research

in collaboration with

Hans Gmünder
GeneData

Drug Discovery Technology
April 15-19, 2002, Stuttgart

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# Agenda

- Uses of DNA micro-arrays in pharmaceutical research
- Case study: Differential gene expression upon induction of apoptosis
- Comparison of physiological with drug-induced apoptosis
- High-throughput functionalisation approach for candidate genes

# Scope of Expression Profiling in Drug Discovery

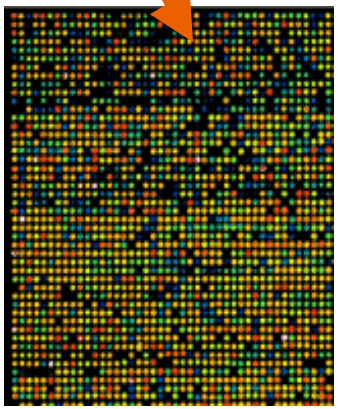
- Genome-wide expression profile of all cell-types and tissues of the human body (“expression atlas” or human transcriptome)
- Expression profiles in all major pharmaceutical animal models
- Comparison of normal with diseased tissue (human as well as animal models)
- Analysis of perturbation of pattern/pathways in disease vs. normal or by drugs
- Generation of complete signal transduction pathways

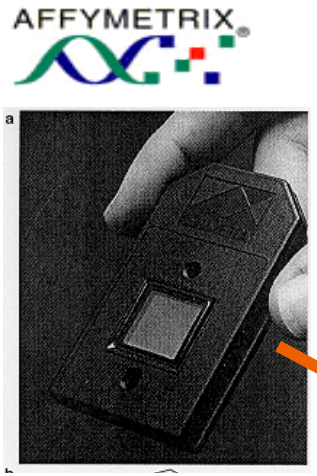
# Applications of Expression Profiling in Drug Discovery

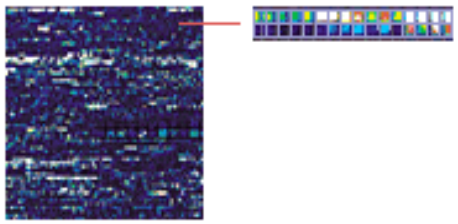
- Target discovery
- Pathway delineation
- Understanding of the disease
- Drug candidate profiling - mechanism-driven versus side-effect profile
- Safety profile of drug candidates

… and many potential applications during drug development

# Technologies:
## High Density Microarrays from Incyte Genomics or AFFYMETRIX





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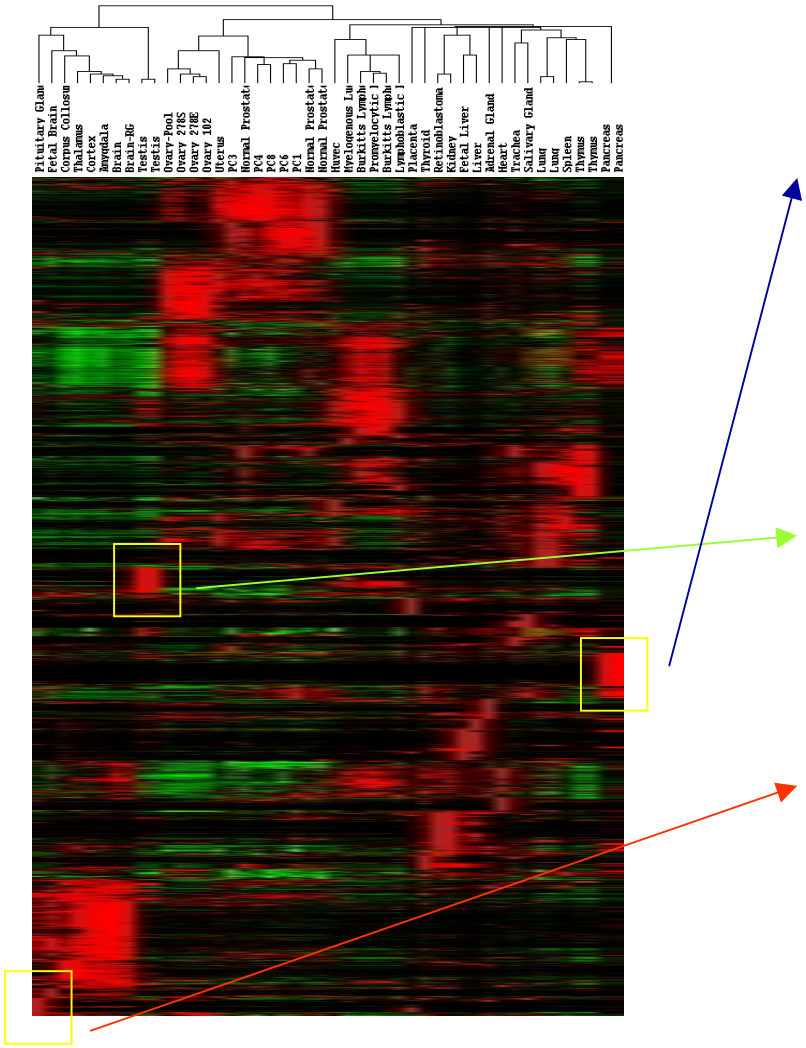
# Uses in Pharmaceutical Research:
## Tissue Reference Database: "Gene Expression Atlas"

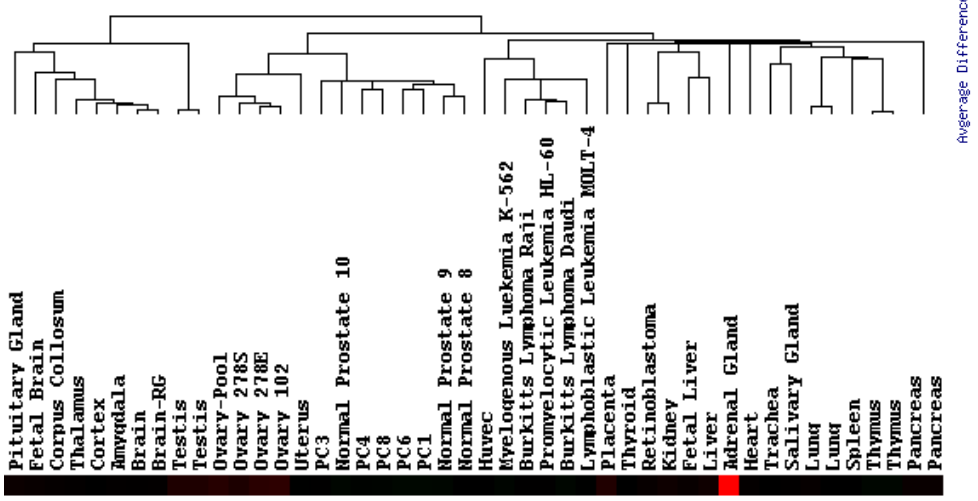


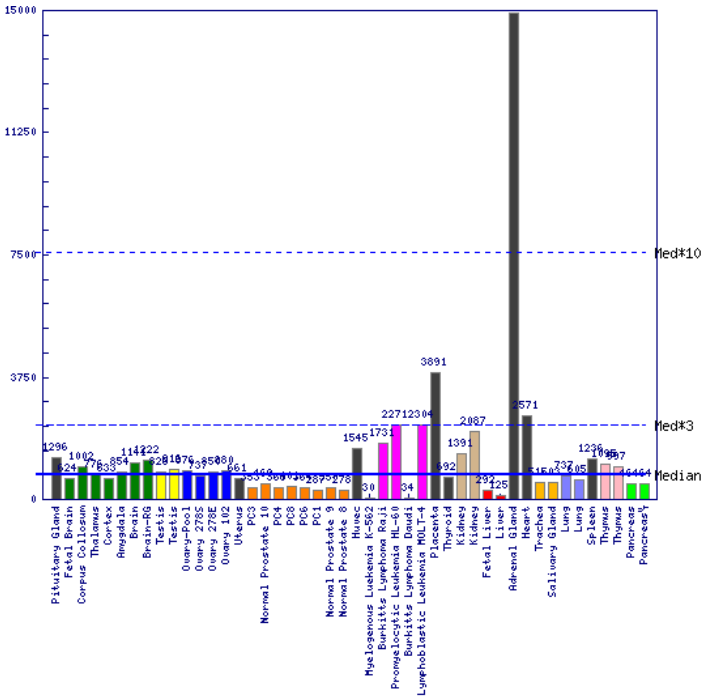

### Pancreas
Pancreatic Lipase
Pancreatic Zymogen
Bile salt activated lipase
Chymotrypsinogen
Chymotrypsin
Etc. etc.

### Testis
Acrosin trypsin inhibitor
Gage 2, Gage 4, Gage 5, Gage 6, Gage 7
Y-box binding protein 1
TubA2, alpha tubulin
Testis specific ankyrin containing protein
Protamine 1, Protamine 2

### Pituitary
Growth hormone 1, growth hormone 2
Chorionic gonadotropin
Follicle Stimulating Hormone
ACTH
Prolactin
Secretogranin
Etc. etc.

# Uses in Pharmaceutical Research:
## "Gene Expression Atlas" : Adrenal Gland Specific Expression




p53/HEH epoxide hydrolase
steroidogenic acute regulatory protein
AF017060
cholesterol side-chain cleavage enzyme P450scc
CLA-1 mRNA
aldose reductase
5-aminolevulinate synthase
steroid 17-alpha-hydroxylase
steroid 17-alpha-hydroxylase/17,20 lyase
cytochrome P-450 (11 Beta)
type II 3-beta hydroxysteroid dehydrogenase
cytochrome P-450 (11 Beta)
21-hydroxylase B gene
cytochrome P-450c11
glutathione S-transferase A4-4
KIAA0018
lamin B receptor homolog TM7SF2



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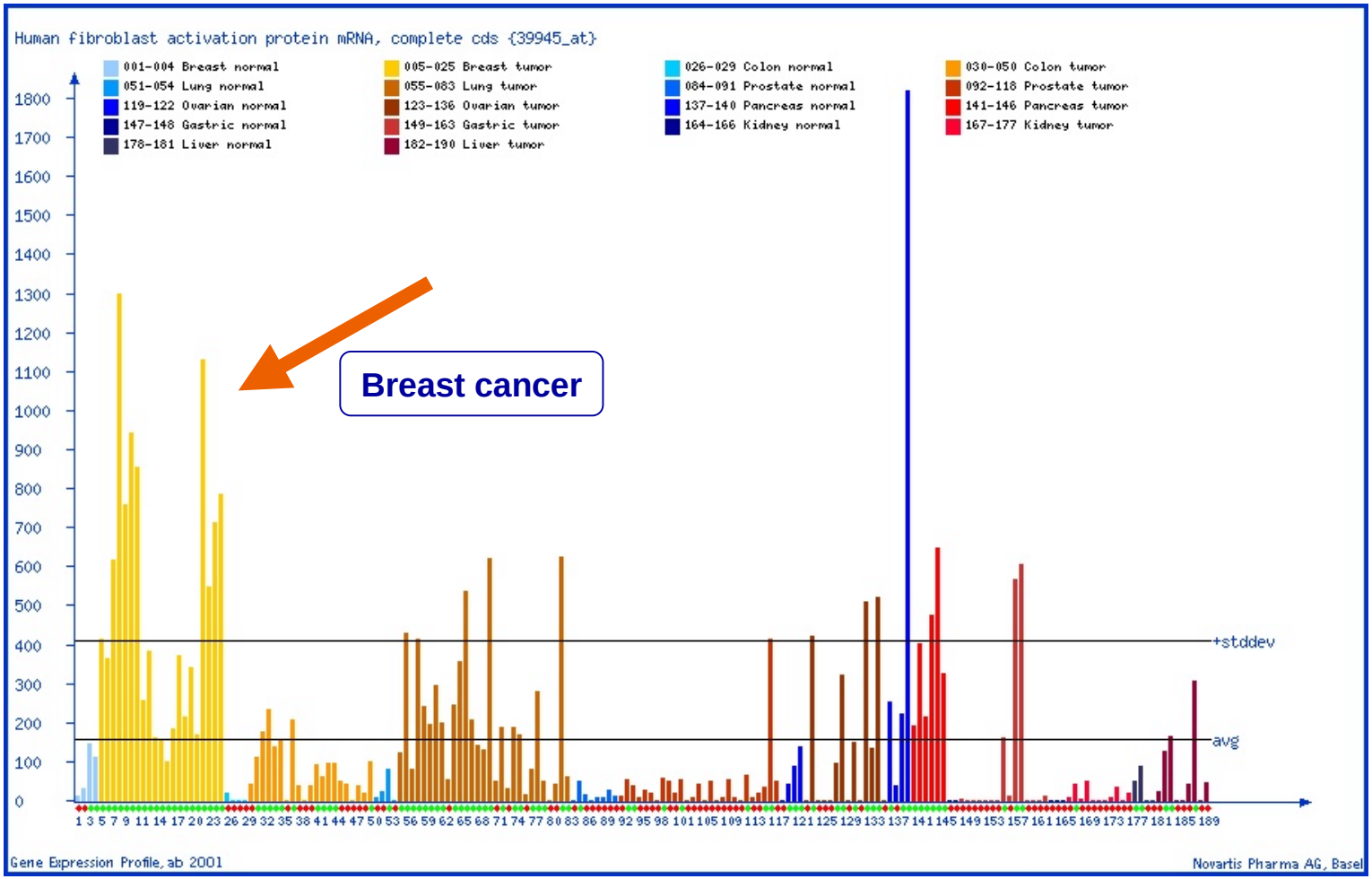
# Uses in Pharmaceutical Research:
## Tumor vs. Normal Tissue on a Gene-by-Gene Basis



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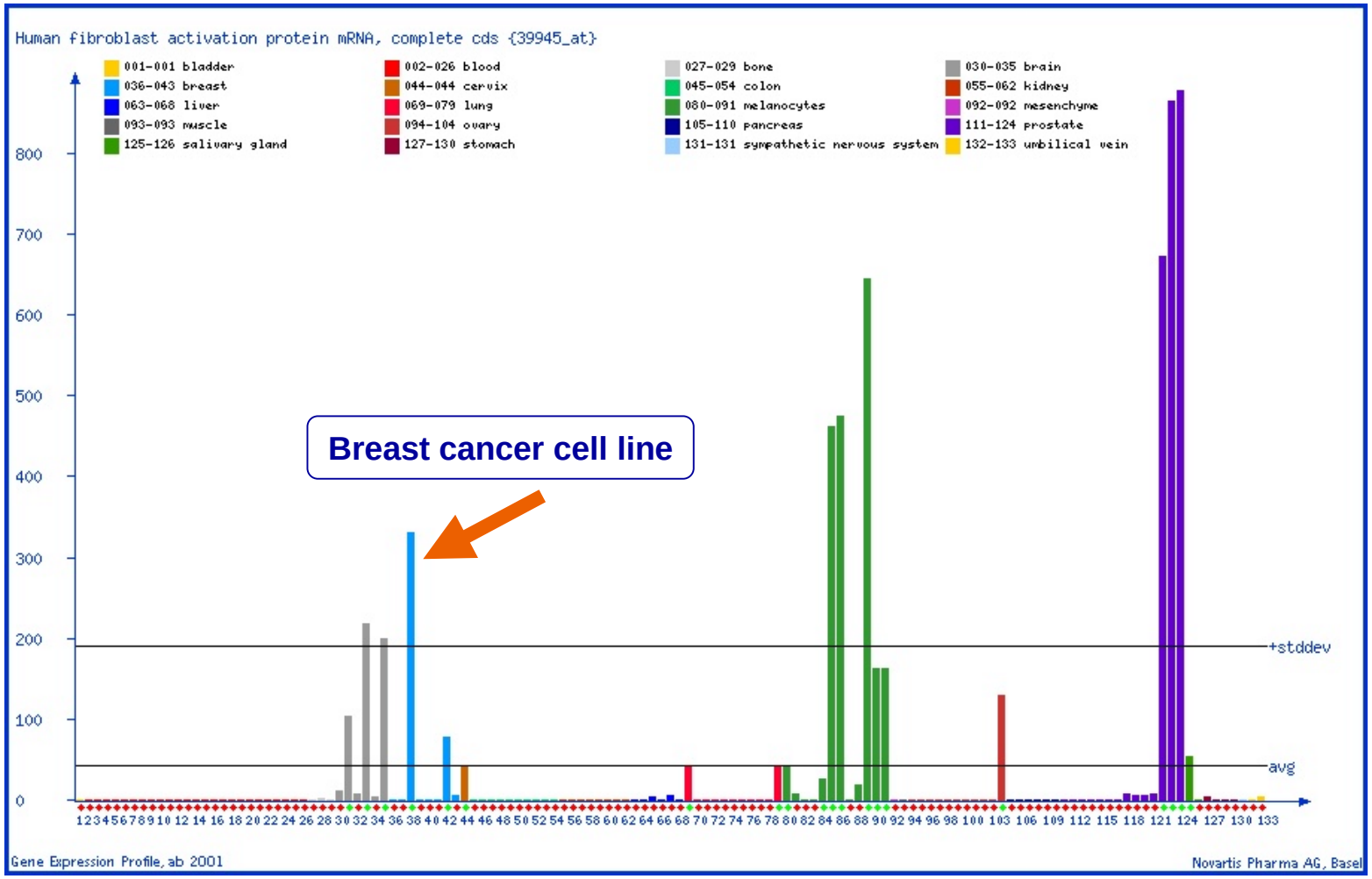
# Uses in Pharmaceutical Research:
## Profiling of the NCI Panel of Cancer Cell Lines



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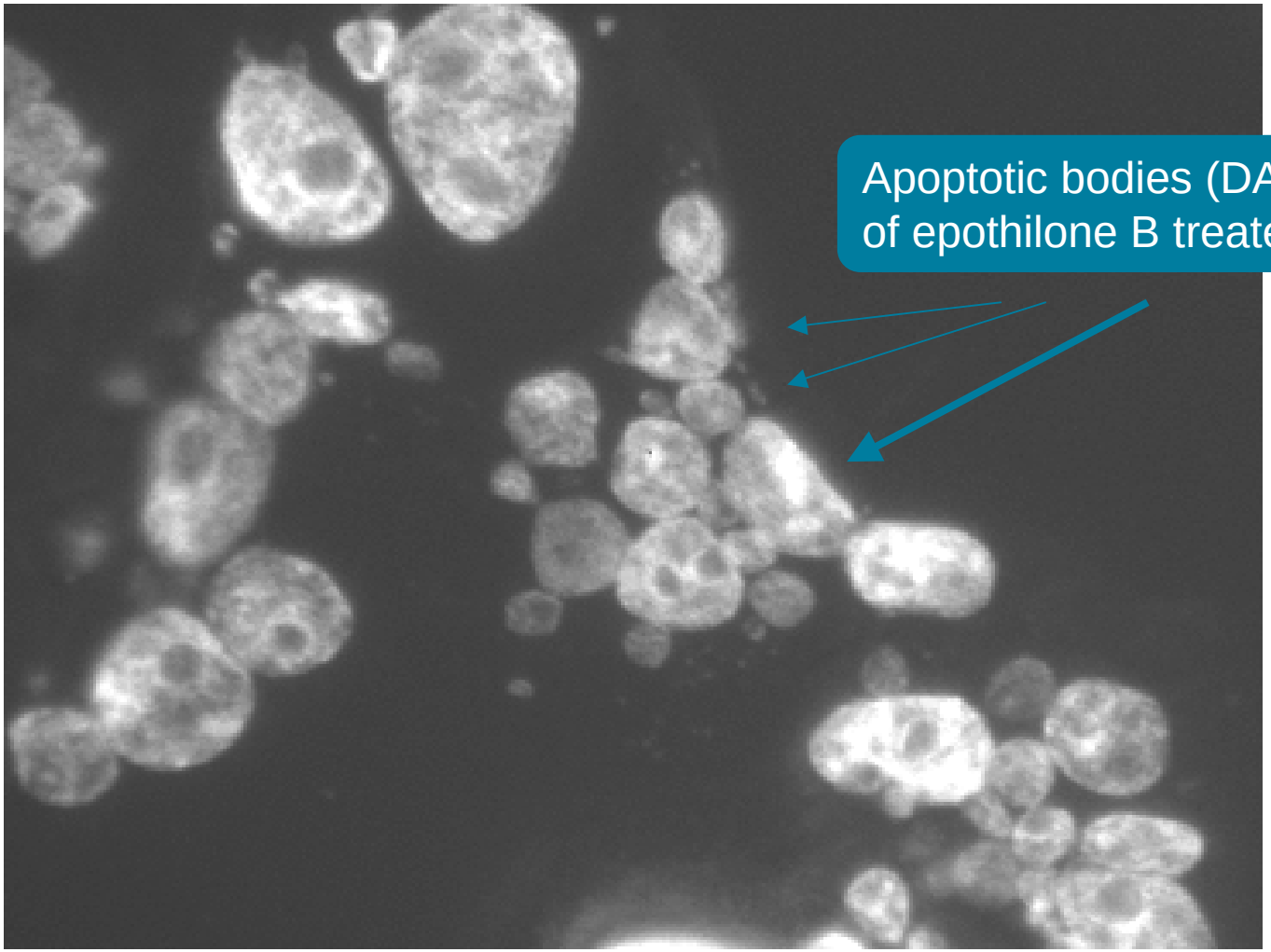
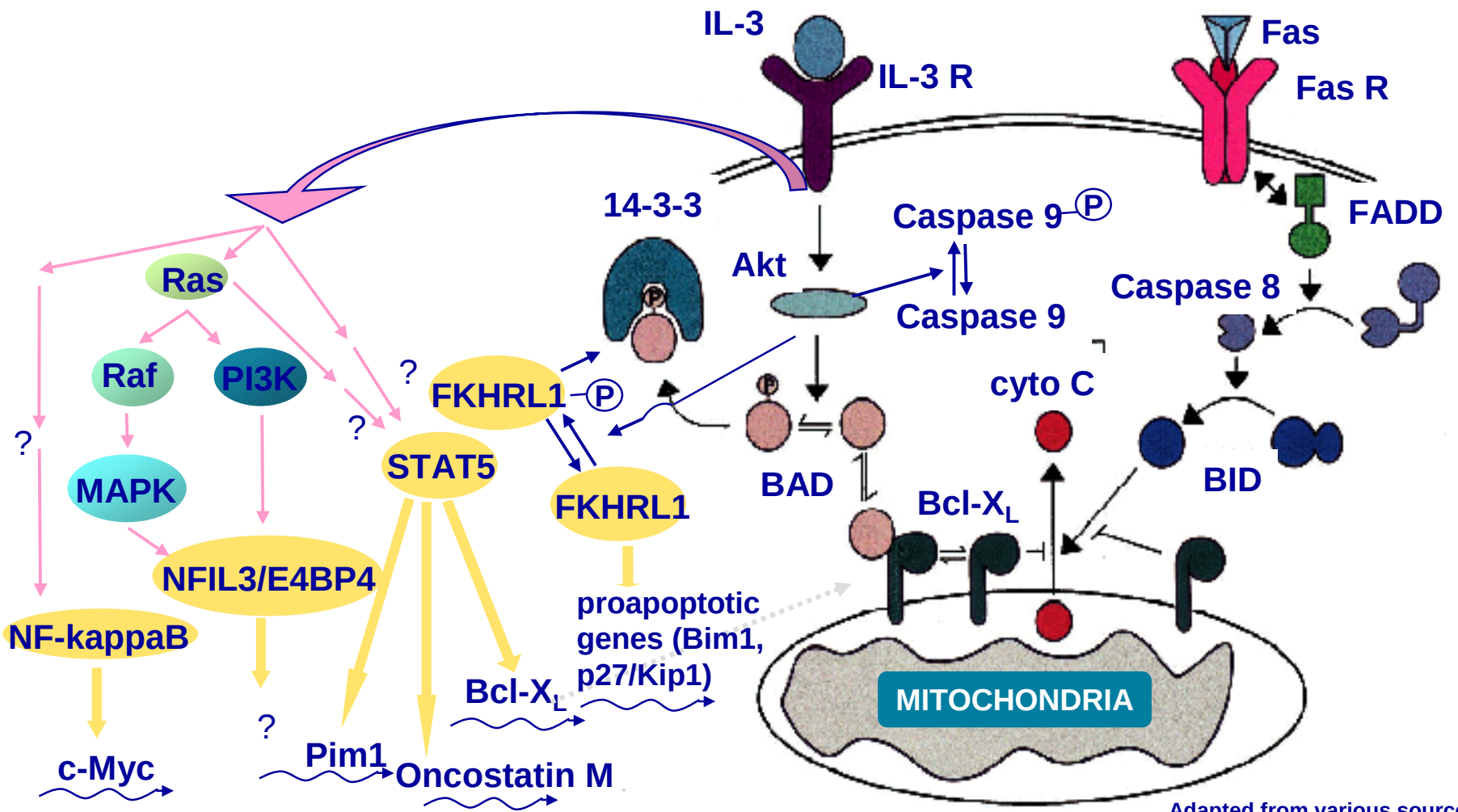
# Case Study Apoptosis
## Complex Biological Process Regulating Tissue Homeostasis



# Case Study Apoptosis

## Elements of Interleukin 3 (IL-3) Signaling in the Context of Apoptosis




Adapted from various sources



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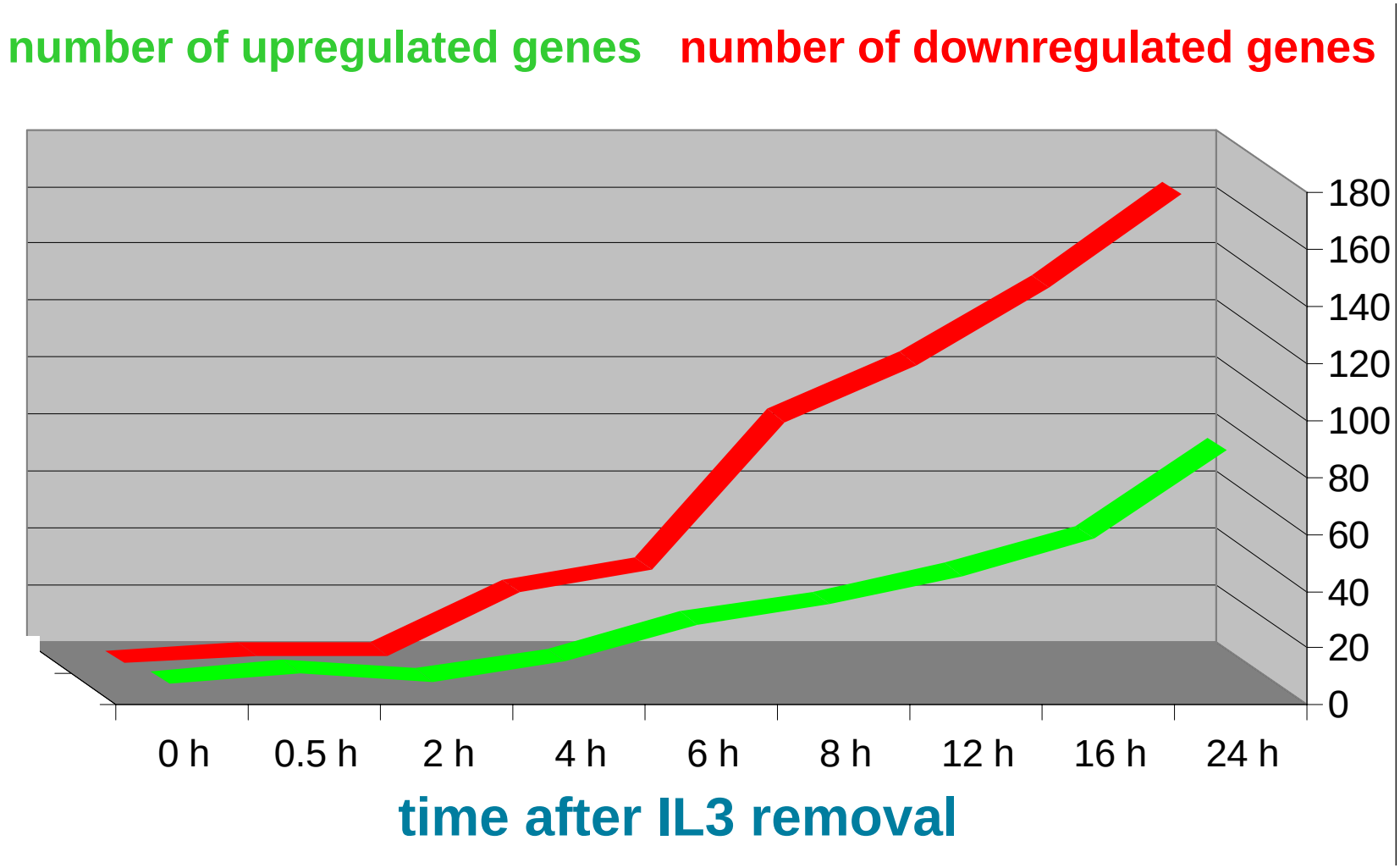
# Case Study Apoptosis:
## Transcriptional Response to IL-3 Deprivation in FL5.12 cells



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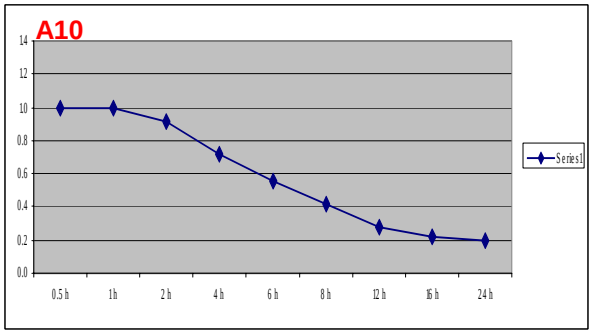
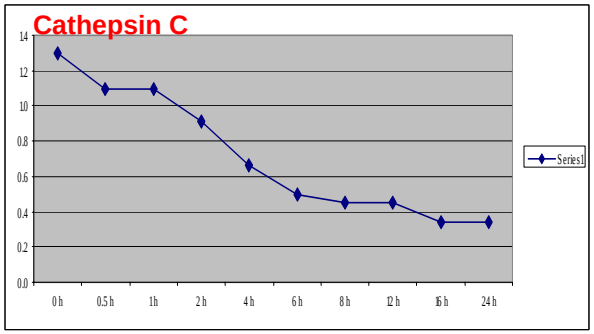
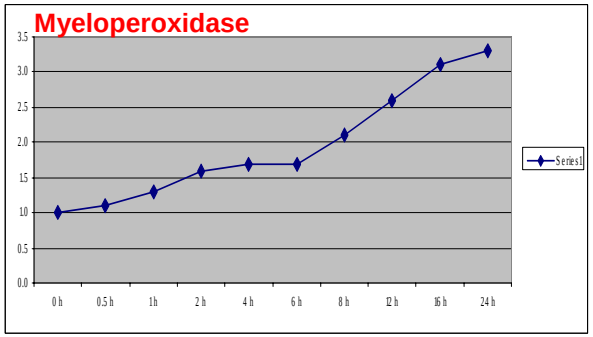
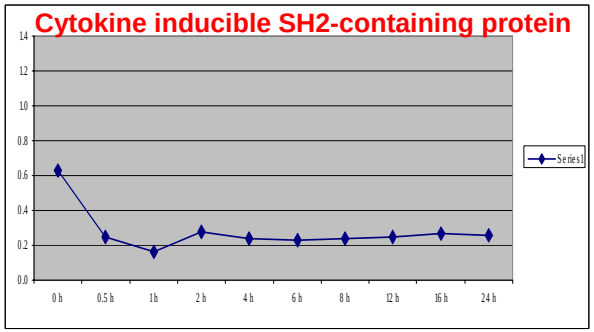
# Case Study Apoptosis

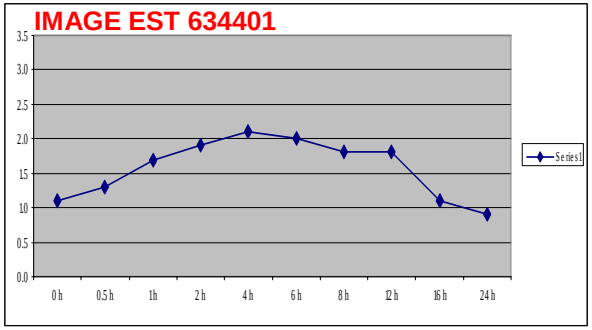
## Time-courses of expression changes are distinct

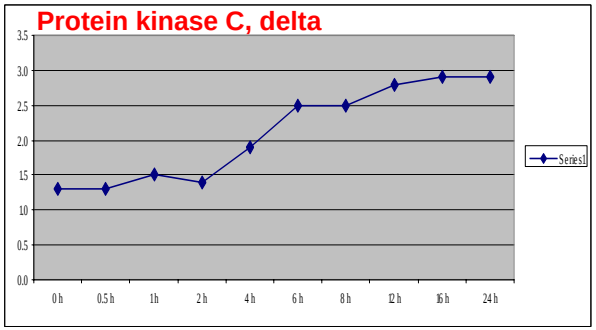


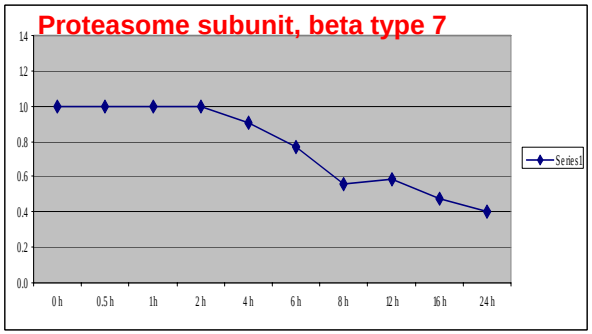



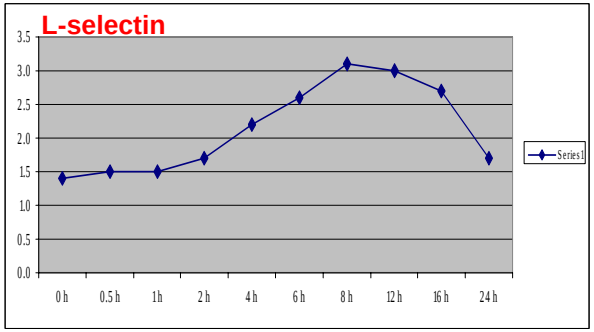



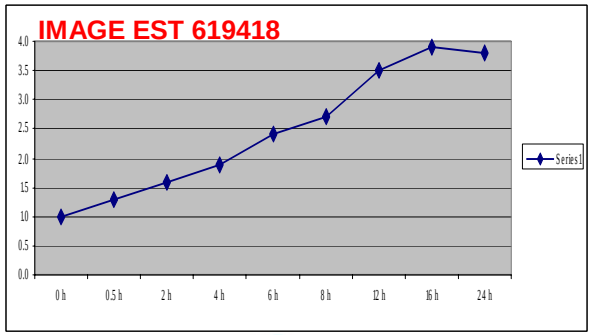



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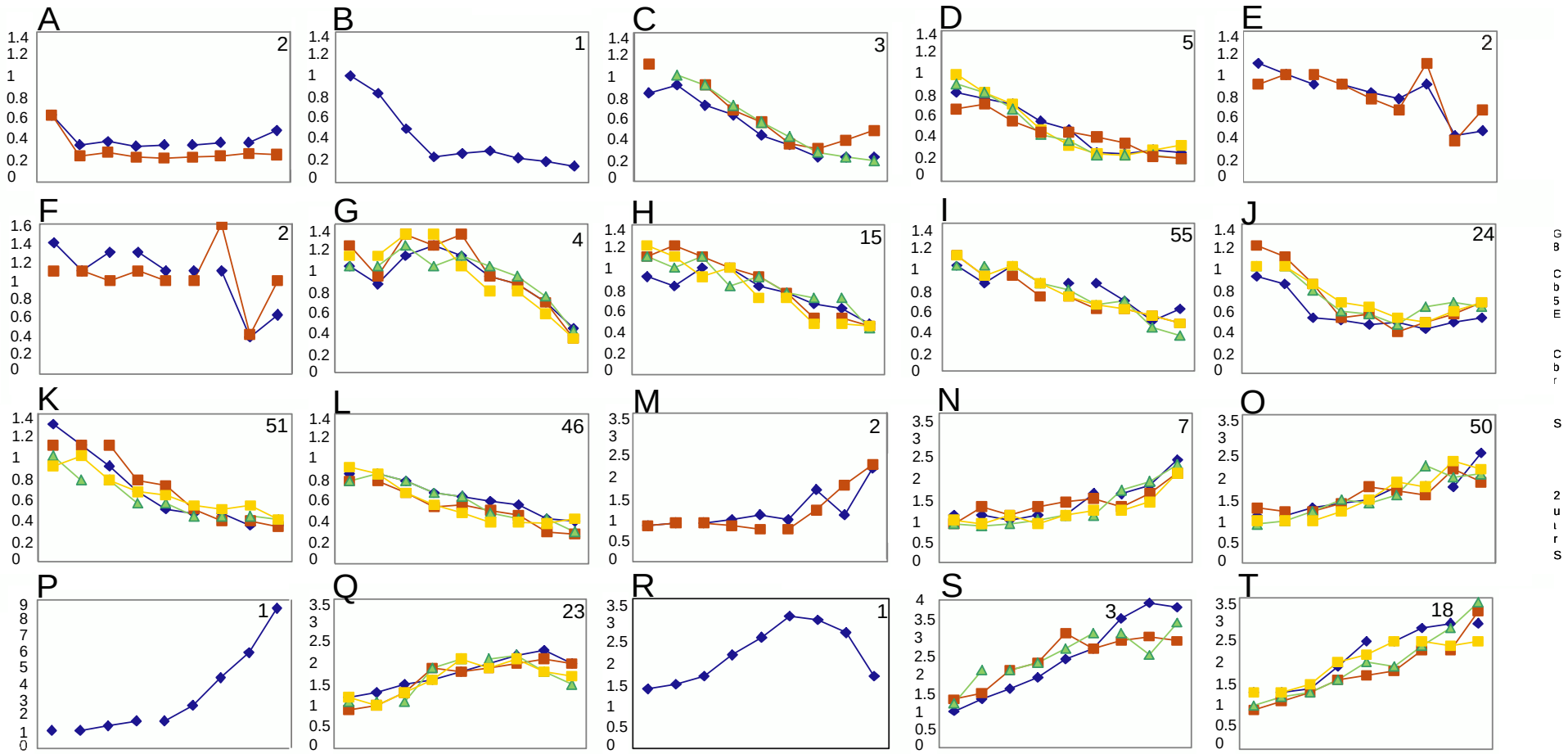
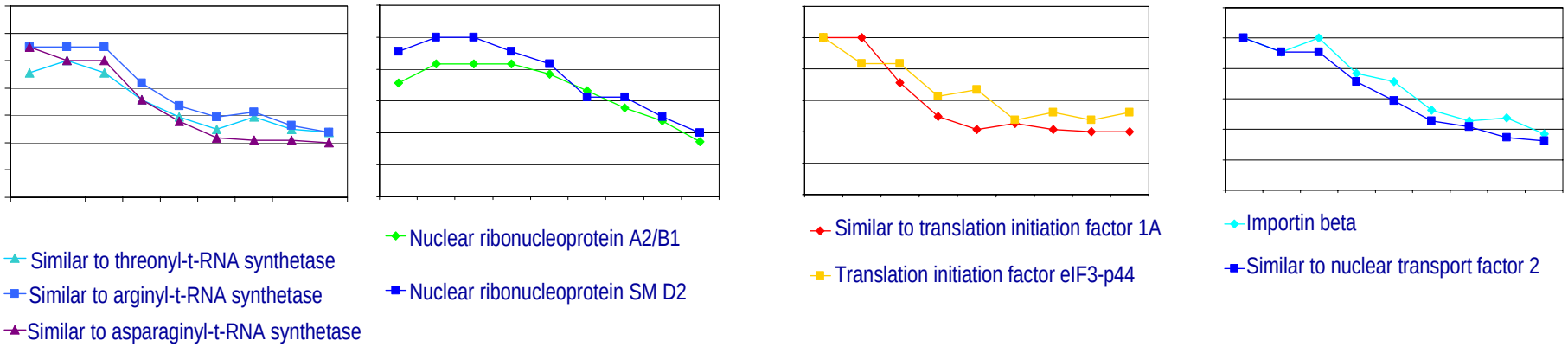
# Case Study Apoptosis

## Hierarchical Clustering of Time-courses



# Case Study Apoptosis

## Cluster Interpretation:
## Similarity in Function Reflected in Similarity of Profile



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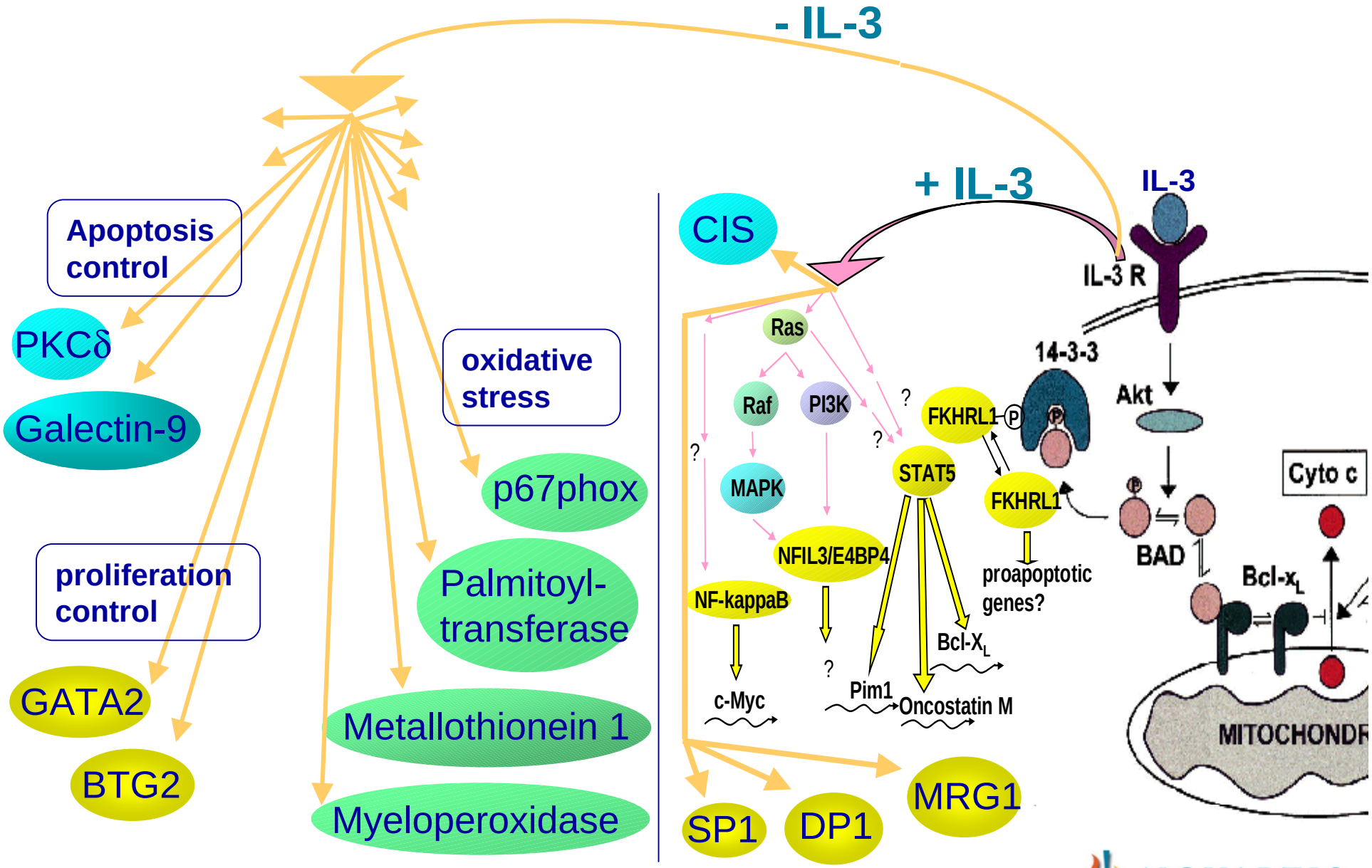
# Case Study Apoptosis
## GO Based Clustering: Biological Process or Objective

Induction of apoptosis
- PKC delta ↑
- Myeloperoxidase ↑
- Carnitine palmitoyltransferase ↑
- c-myc... ↓
- Galectin-9 ↑
- Serglycin? ↑

Cell cycle arrest
- c-myc ↓
- DP1 ↓
- Replication factor CDC46 ↓
- Subunit of RNA Pol 1 ↓
- Splicing factors ↓
- Aminoacid-tRNA synthetases ↓
- GATA-2... ↑

Stress response
- Metallothionein 1 ↑
- PBP74 ↓
- HSP65... ↓

# Apoptosis: New Elements in IL-3 Signaling



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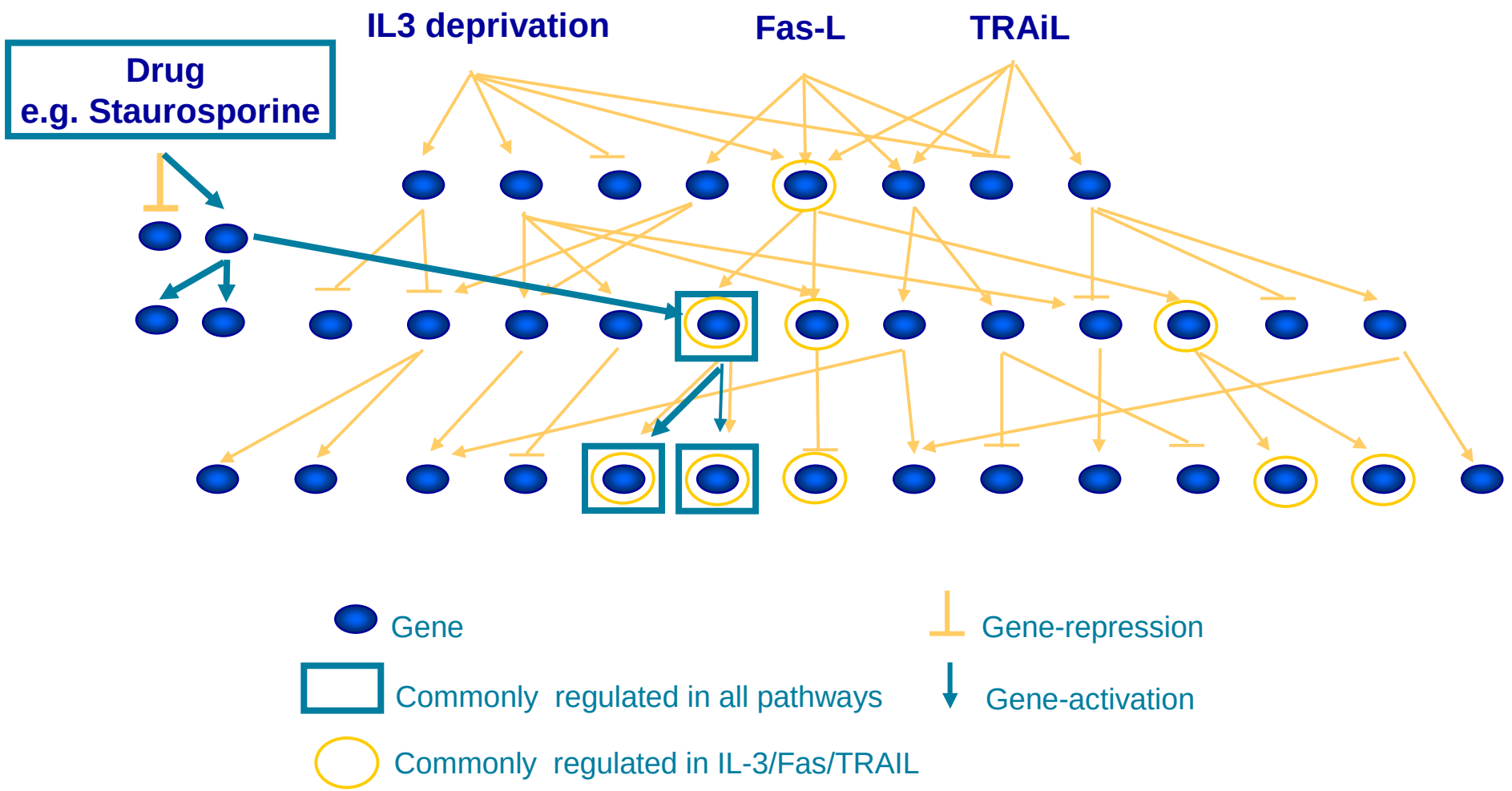
# Comparison of "Physiological" with Drug-Induced Apoptosis



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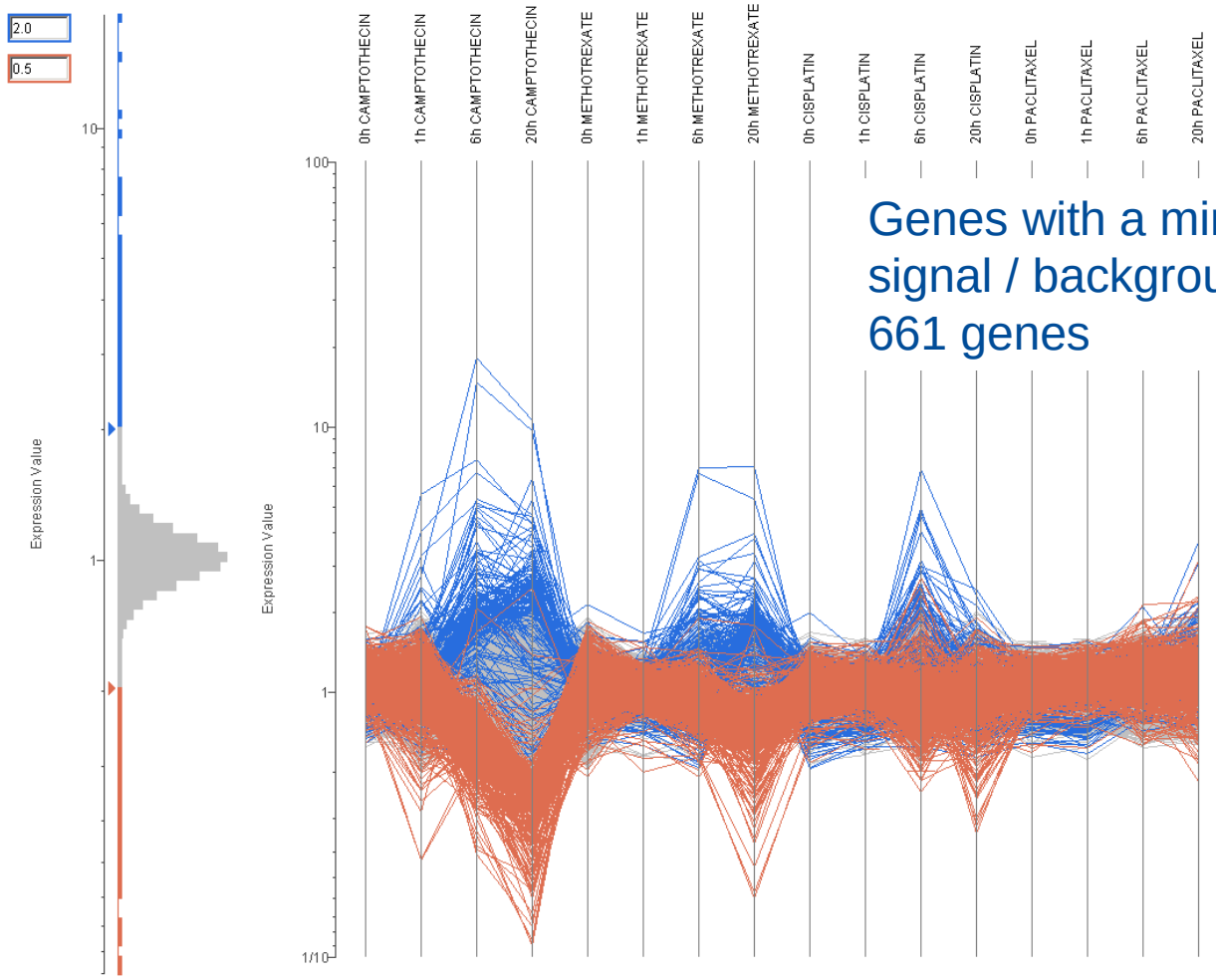
# Drug-induced Apoptosis
## Different mode of action of cancer drugs

- Cisplatin **binds to DNA and interferes with the repair mechanism.**
- Paclitaxel (taxol) **promotes the assembly of microtubules from tubulin dimers and stabilizes microtubules by preventing depolymerization resulting in the inhibition of the normal dynamic reorganization of the microtubule network that is required for vital interphase and mitotic cellular functions.**
- Camptothecin **and its derivatives inhibit DNA Topoisomerase I by stabilizing a covalent reaction intermediate termed the cleavable complex .**
- **Folate is involved in the synthesis, repair, and functioning of DNA and a deficiency of folate may result in damage to DNA.** Methotrexate**, an inhibitor of the dihydrofolate reductase, limits the activity of enzymes that need folate.**

# Drug-induced Apoptosis
## Expression value filtering




Genes with a minimum signal / background ratio >2 selected 661 genes



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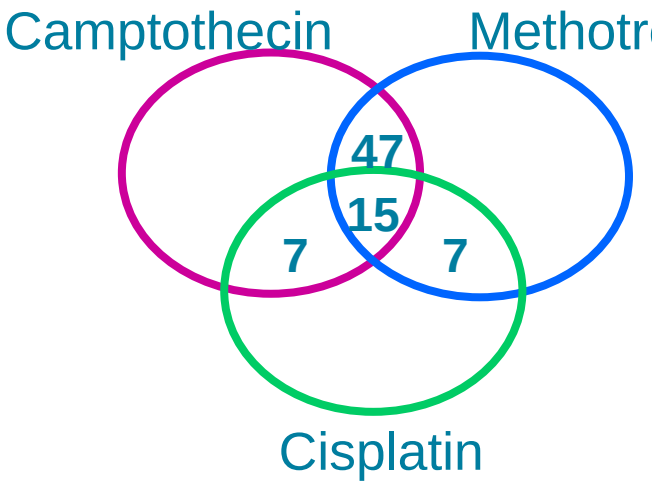
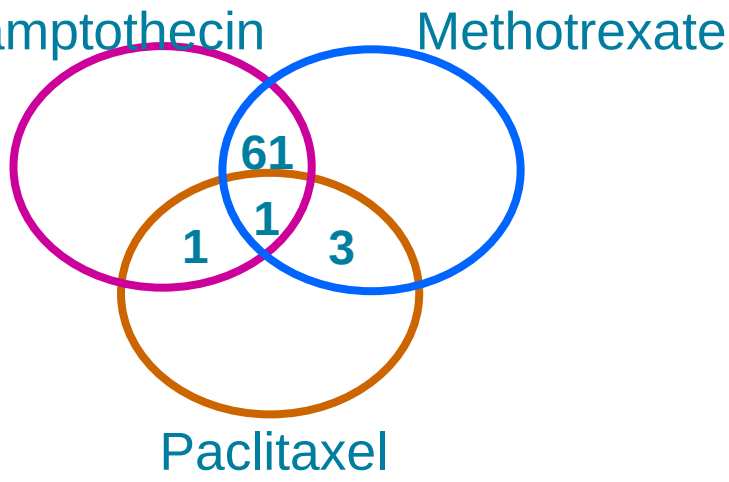
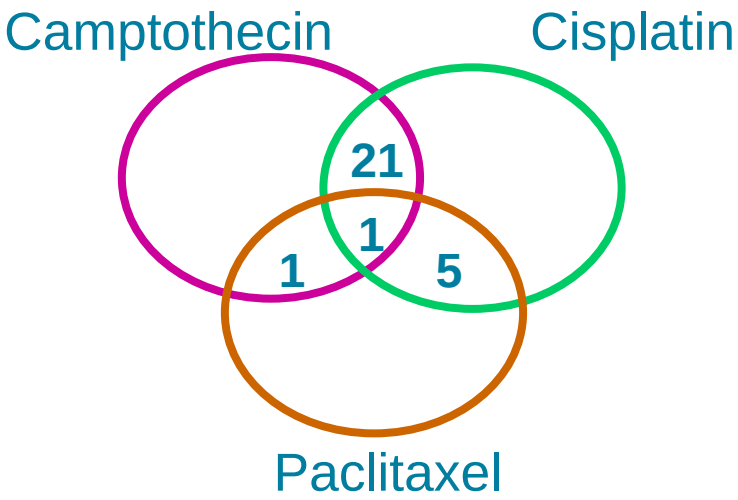
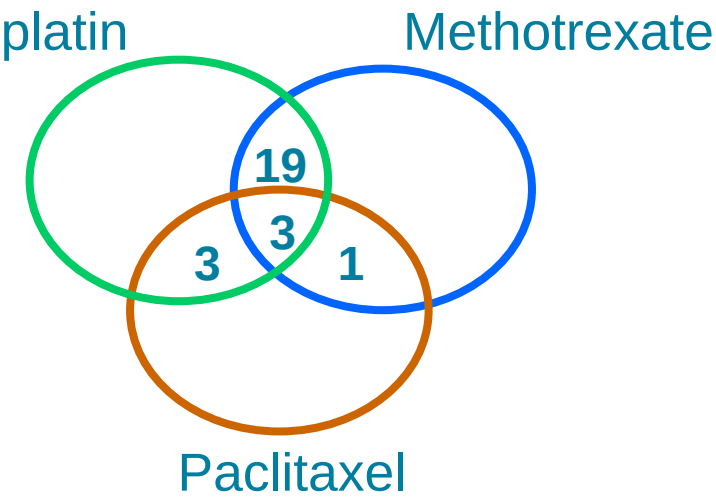
# Drug-induced Apoptosis

## Intersections of Genes Indicate Similarities in Pathways



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# Drug-induced Apoptosis

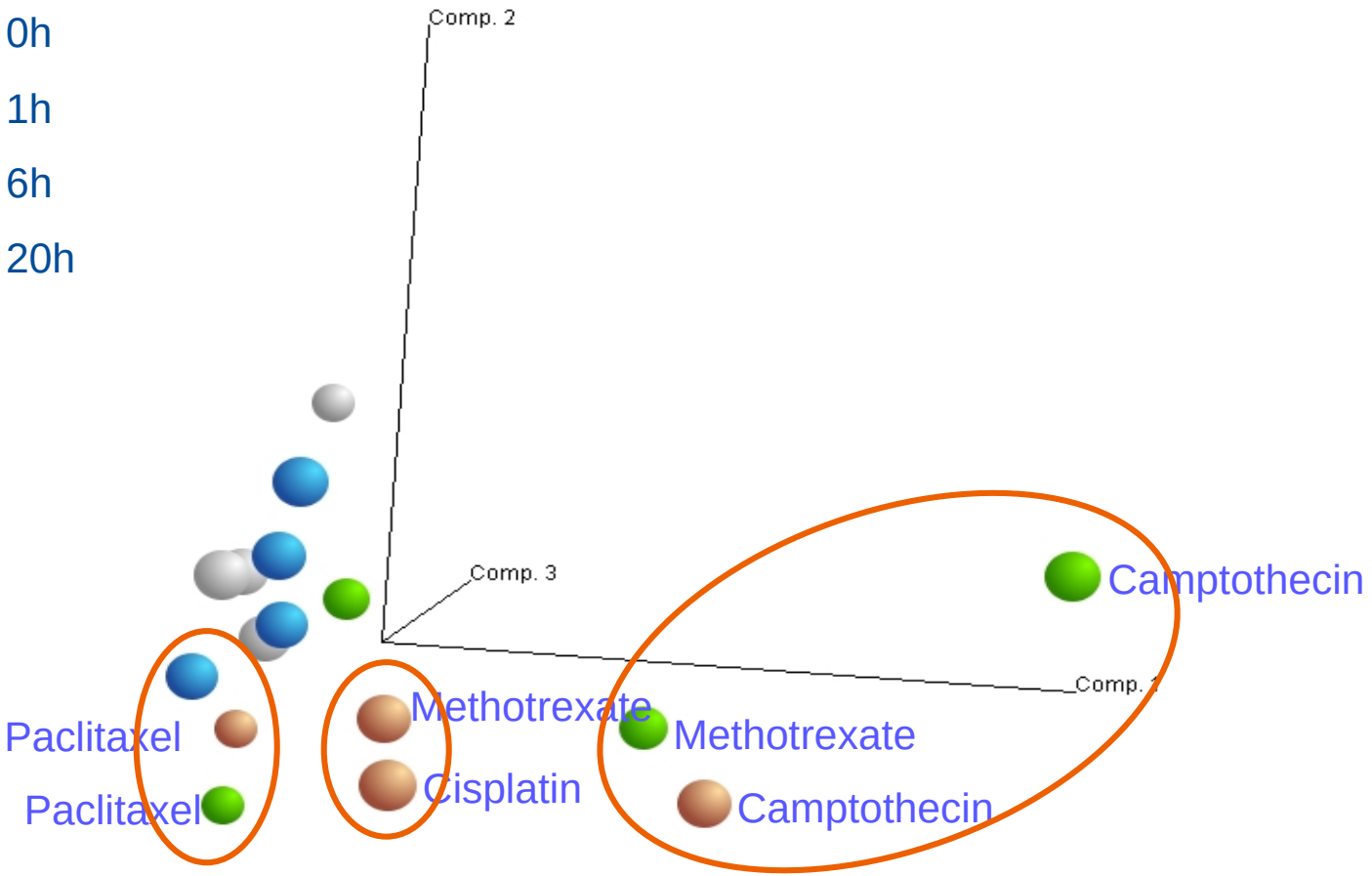
## Principal Components Analysis: 3D view of components 1, 2 and 3 lead to identification of groups

White = 0h

Blue = 1h

Brown = 6h

Green = 20h



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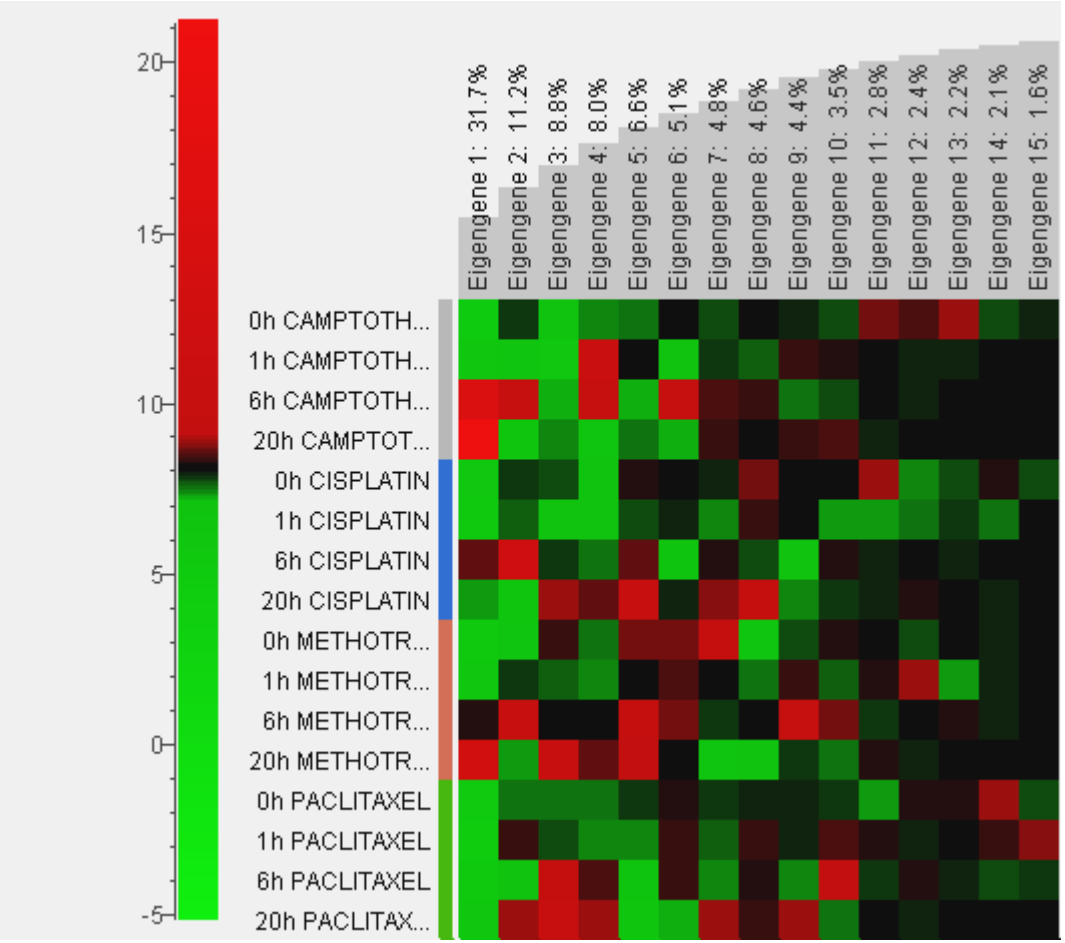
# Drug-induced Apoptosis
## Experiments Projected on Principal Components




The experiments are projected onto each component (axis) found by the algorithm.

The projection values for each experiment are color coded (red, green).

The Eigengene can be interpreted as a gene profile that shows a specific characteristic of the data set.

Eigengenes 1 to 4 explain 60 % of variance.



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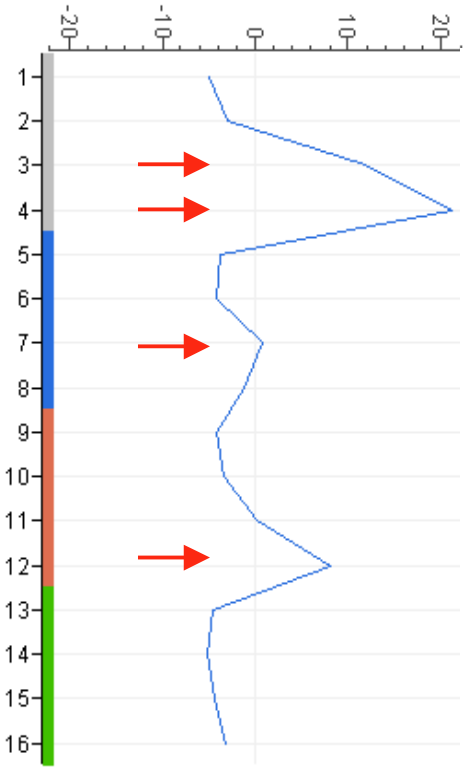
# Drug-induced Apoptosis

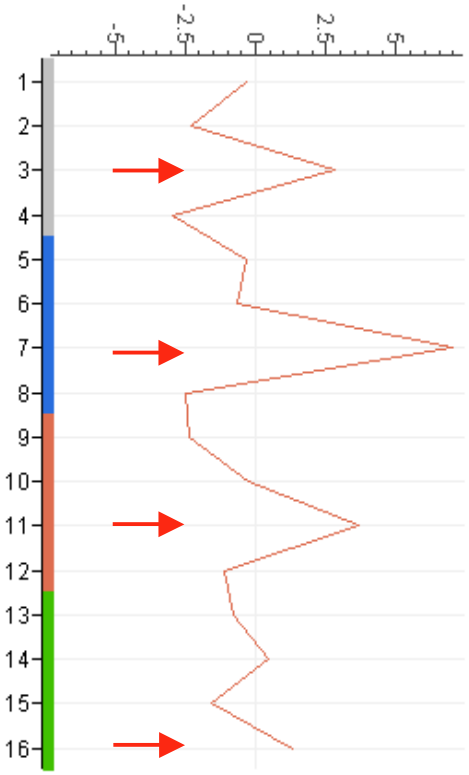
## Eigenprofiles 1 and 2

### Projection of Eigengene 1




0h Camptothecin
1h Camptothecin
6h Camptothecin
20h Camptothecin
0h Cisplatin
1h Cisplatin
6h Cisplatin
20h Cisplatin
0h Methotrexate
1h Methotrexate
6h Methotrexate
20h Methotrexate
0h Paclitaxel
1h Paclitaxel
6h Paclitaxel
20h Paclitaxel

### Projection of Eigengene 2




0h Camptothecin
1h Camptothecin
6h Camptothecin
20h Camptothecin
0h Cisplatin
1h Cisplatin
6h Cisplatin
20h Cisplatin
0h Methotrexate
1h Methotrexate
6h Methotrexate
20h Methotrexate
0h Paclitaxel
1h Paclitaxel
6h Paclitaxel
20h Paclitaxel

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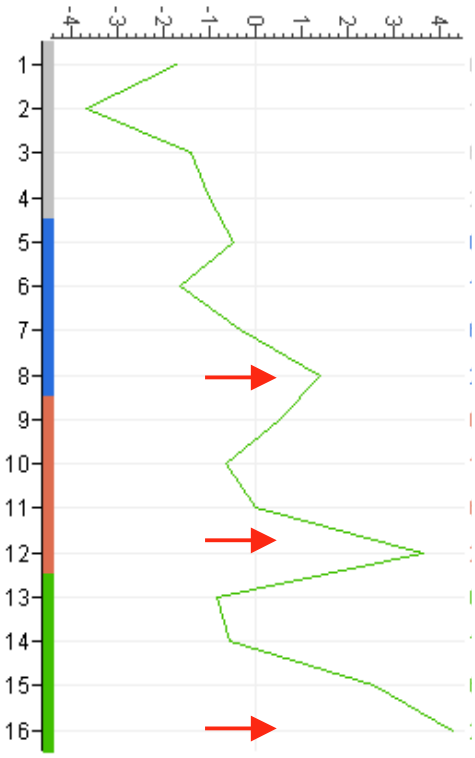
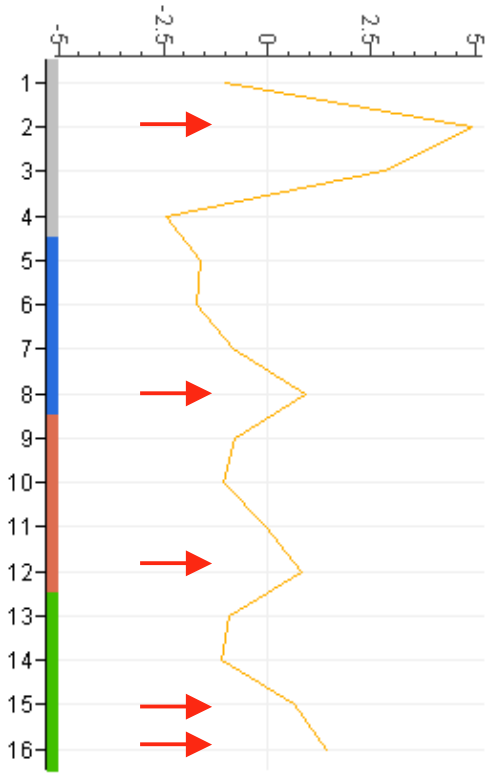
# Drug-induced Apoptosis
## Eigenprofiles 3 and 4

### Projection of Eigengene 3




0h Camptothecin
1h Camptothecin
6h Camptothecin
20h Camptothecin
0h Cisplatin
1h Cisplatin
6h Cisplatin
20h Cisplatin
0h Methotrexate
1h Methotrexate
6h Methotrexate
20h Methotrexate
0h Paclitaxel
1h Paclitaxel
6h Paclitaxel
20h Paclitaxel

### Projection of Eigengene 4




0h Camptothecin
1h Camptothecin
6h Camptothecin
20h Camptothecin
0h Cisplatin
1h Cisplatin
6h Cisplatin
20h Cisplatin
0h Methotrexate
1h Methotrexate
6h Methotrexate
20h Methotrexate
0h Paclitaxel
1h Paclitaxel
6h Paclitaxel
20h Paclitaxel

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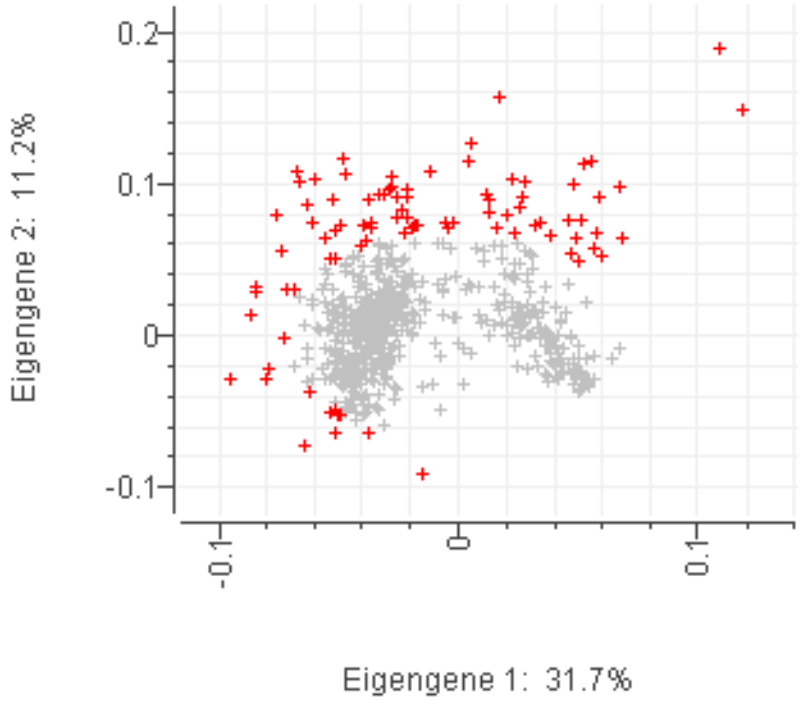
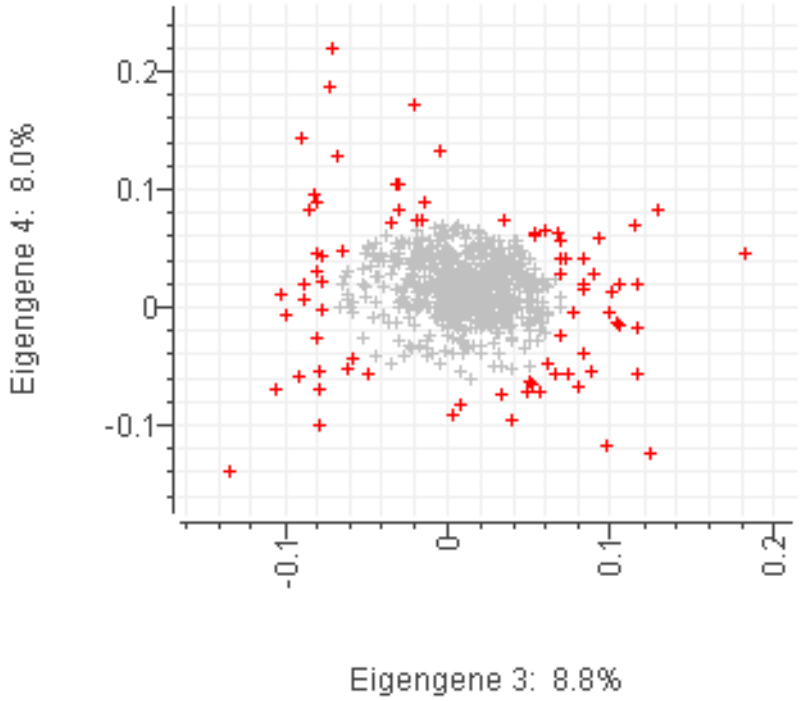
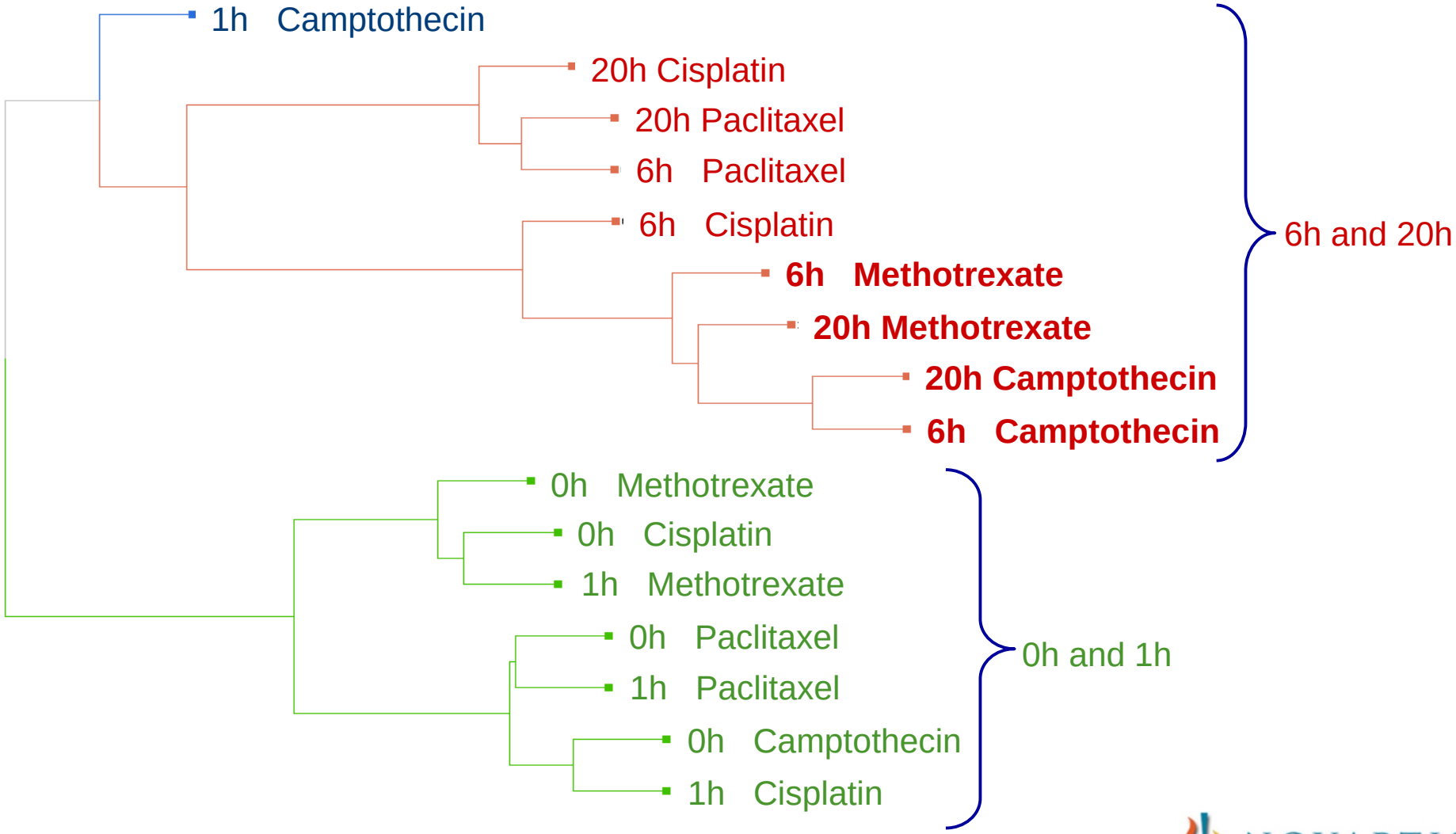
# Drug-induced Apoptosis
## Gene Loadings




The loadings view's 2D plot represents the projection of genes in two components.
The genes are projected on each component (axis) found by the algorithm.
147 genes contribute the most to the 4 components.

# Drug-induced Apoptosis
## Hierarchical Clustering



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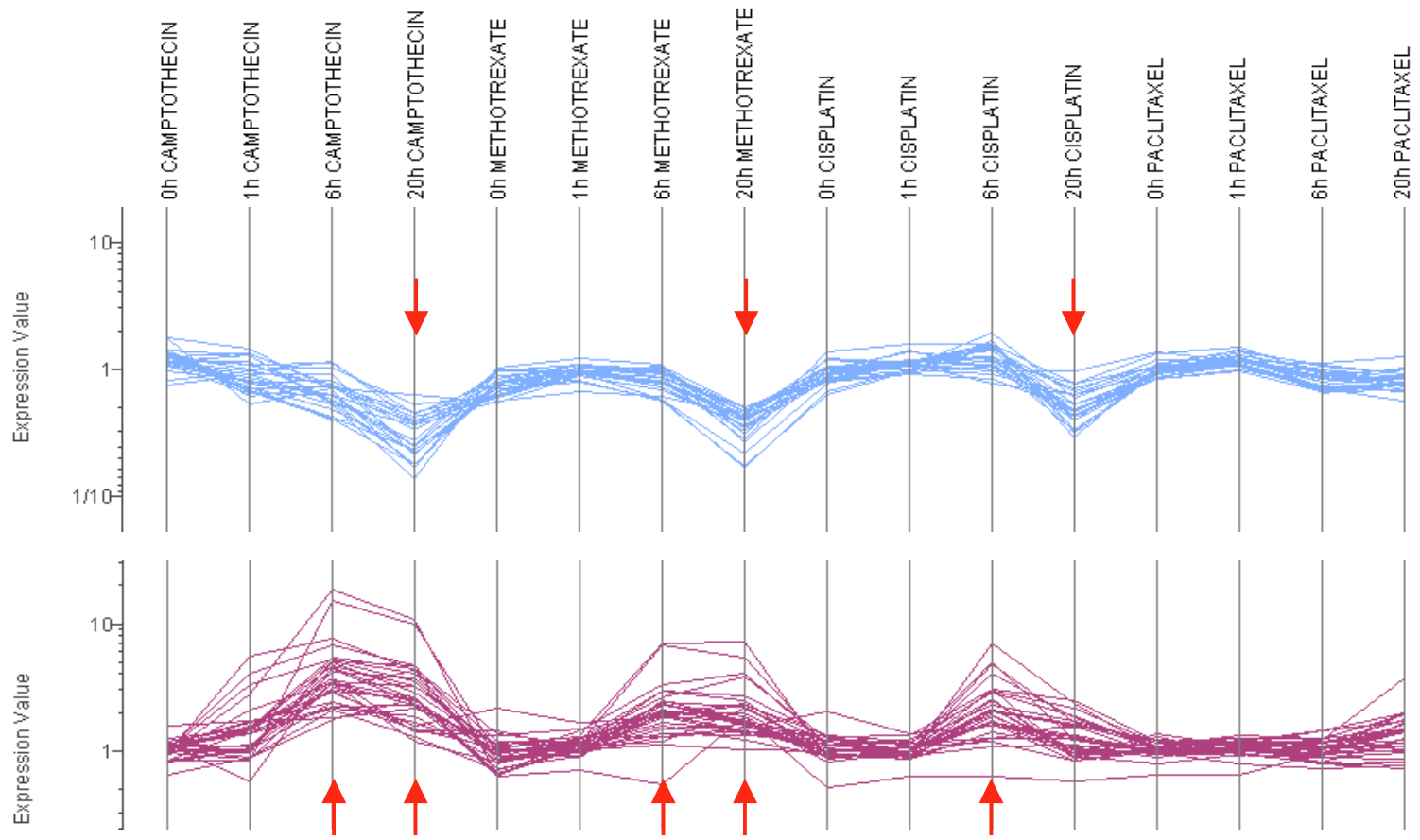
# Drug-induced Apoptosis
## SOM Partitioning



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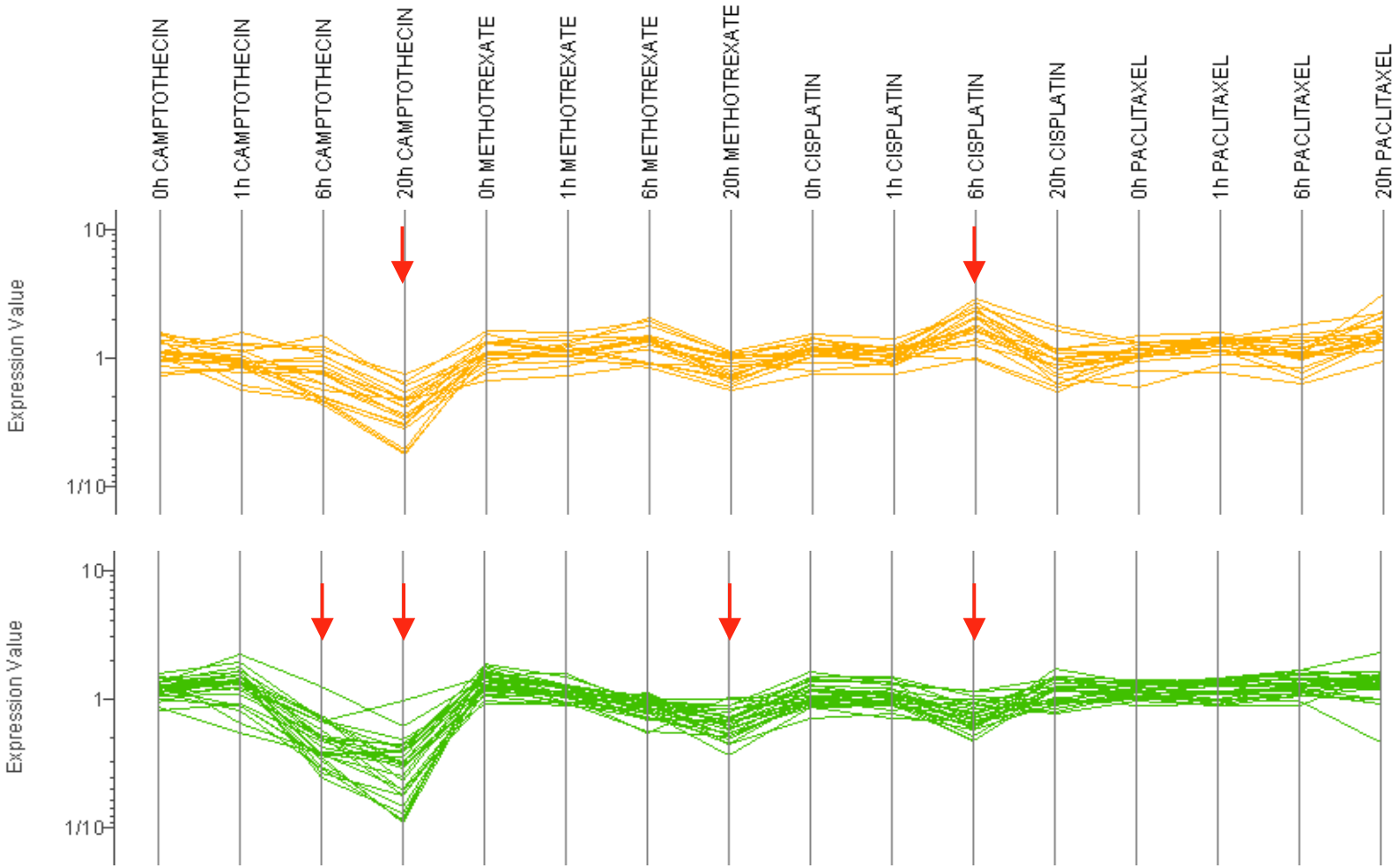
# Drug-induced Apoptosis
## SOM Partitioning



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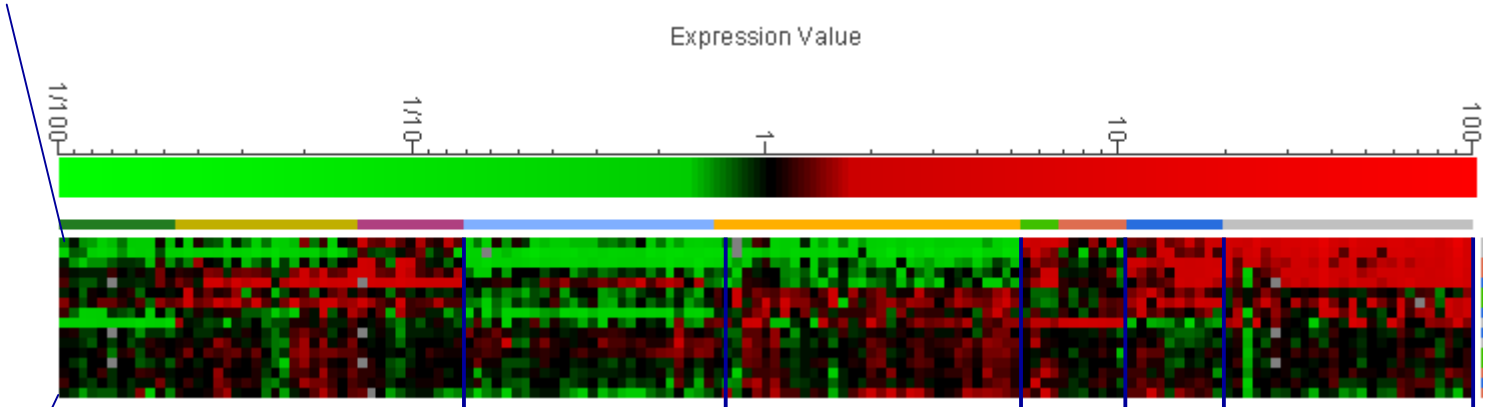
# Drug-induced Apoptosis
## SOM Partitioning Tile Display

**Top**

6h Camptothecin
20h Camptothecin
20h Methotrexate
6h Methotrexate
6h Cisplatin
6h Paclitaxel
20h Paclitaxel
20h Cisplatin
1h Camptothecin
1h Cisplatin
0h Camptothecin
1h Paclitaxel
0h Paclitaxel
1h Methotrexate
0h Cisplatin
0h Methotrexate

**Bottom**




| down | down | up | up |
|---|---|---|---|
| 6h Camptothecin | 6h Camptothecin | 20h Methotrexate | 6h Camptothecin |
| 20h Camptothecin | 20h Camptothecin | 6h Methotrexate | 20h Camptothecin |
| 20h Methotrexate | 20h Methotrexate | 6h Cisplatin | 20h Methotrexate |
| 20h Cisplatin | 6h Cisplatin | 20h Paclitaxel | 6h Methotrexate |
| | | | 6h Cisplatin |
| | | | 20h Paclitaxel |
| | | | 20h Cisplatin |



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# Drug-induced Apoptosis
## Upregulated Gene Cluster 1, snapshot

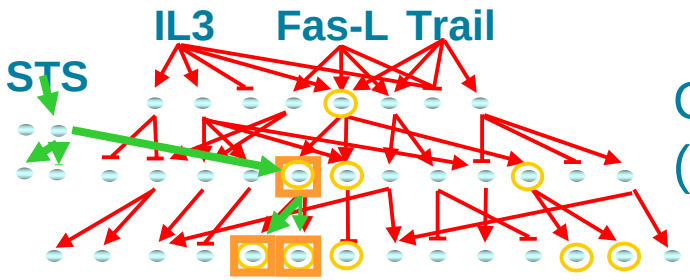
| Gene Symbol and Name | 6h Camptothecin | 20h Camptothecin | 6h Methotrexate | 20h Methotrexate | 6h Cisplatin | 20h Cisplatin | 20h Paclitaxel |
|---|---|---|---|---|---|---|---|
| Btg2 B-cell translocation gene 2, anti-proliferative | 18.2 | 10.6 | 7.0 | 7.1 | 4.8 | 1.7 | 1.7 |
| B-cell translocation gene 2, anti-proliferative | 2.2 | 2.3 | 2.3 | 1.8 | 2.1 | 0.9 | 0.8 |
| Zfp36l1: zinc finger protein 36, C3H type-like 1 | 5.2 | 4.0 | 2.3 | 1.6 | 1.9 | 1.0 | 1.1 |
| Casp11: caspase 11 | 3.3 | 1.2 | 2.3 | 1.7 | 1.6 | 0.8 | 1.4 |
| Cln2: ceroid-lipofuscinosis, neuronal 2 | 3.1 | 2.4 | 1.7 | 1.4 | 1.9 | 1.0 | 1.8 |
| Ccng: cyclin G | 3.4 | 3.2 | 2.6 | 3.8 | 2.9 | 2.1 | 1.9 |
| Cdkn1a: cyclin-dependent kinase inhibitor 1A (P21) | 5.0 | 3.5 | 3.2 | 4.0 | 2.8 | 1.8 | 2.0 |
| Ei24: etoposide induced 2.4 mRNA | 5.4 | 4.4 | 2.9 | 2.7 | 3.0 | 2.4 | 1.9 |
| Hba: hemoglobin alpha chain complex | 2.8 | 1.5 | 1.8 | 1.3 | 3.0 | 0.9 | 0.7 |
| Lpin1: lipin 1 | 4.4 | 2.6 | 2.0 | 1.7 | 2.1 | 1.0 | 1.4 |
| Man2a1: mannosidase 2, alpha 1 | 1.8 | 2.1 | 1.3 | 1.5 | 1.5 | 1.3 | 0.8 |
| Myd88: myeloid differentiation primary response gene 88 | 2.4 | 1.6 | 1.3 | 1.3 | 1.3 | 1.6 | 1.4 |
| Pmm1: phosphomannomutase 1 | 3.3 | 2.3 | 2.0 | 1.4 | 2.3 | 1.7 | 1.4 |
| Pvrl4: poliovirus receptor-related 4 | 5.0 | 4.6 | 2.4 | 2.2 | 1.7 | 1.0 | 1.2 |
| Teap-pending: thymus expressed acidic protein | 14.8 | 9.7 | 6.6 | 5.3 | 6.9 | 2.3 | 3.7 |
| Tob1: transducer of ErbB-2.1 | 7.5 | 4.3 | 2.9 | 2.5 | 4.0 | 1.8 | 1.9 |
| Txnip: thioredoxin interacting protein | 4.4 | 2.3 | 2.0 | 2.3 | 2.5 | 0.9 | 1.6 |

# Drug-induced Apoptosis
## Downregulated Gene Cluster 2, snapshot

| Gene Symbol and Name | 6h Camptothecin | 20h Camptothecin | 20h Methotrexate | 20h Cisplatin |
|---|---|---|---|---|
| Aldo1: aldolase 1, A isoform | 0.7 | 0.2 | 0.3 | 0.8 |
| Anxa2: annexin A2 | 0.6 | 0.3 | 0.3 | 0.3 |
| Anxa4: annexin A4 | 0.6 | 0.3 | 0.5 | 0.7 |
| Bnip3: BCL2/adenovirus E1B 19 kDa-interacting protein 1, NIP3 | 0.4 | 0.2 | 0.2 | 0.3 |
| Bnip3l: BCL2/adenovirus E1B 19 kDa-interacting protein 3-like | 0.6 | 0.2 | 0.4 | 0.4 |
| Csrp3: cysteine-rich protein 3 | 0.5 | 0.2 | 0.3 | 0.4 |
| Eno1: enolase 1, alpha non-neuron | 0.5 | 0.2 | 0.3 | 0.6 |
| Similar to MIPP65 protein | 0.7 | 0.3 | 0.4 | 0.5 |
| Weakly similar to Ca18 mouse collagen alpha 1(VIII) chain precursor | 0.5 | 0.4 | 0.4 | 0.5 |
| Gapd: glyceraldehyde-3-phosphate dehydrogenase | 0.5 | 0.1 | 0.3 | 0.6 |
| Gys3: glycogen synthase 3, brain | 0.8 | 0.4 | 0.5 | 0.5 |
| Hig1-pending: hypoxia induced gene 1 | 0.8 | 0.2 | 0.3 | 0.5 |
| Metallothionein 1 (Mt1), mRNA | 0.9 | 0.2 | 0.4 | 0.5 |
| Egln1: EGL nine homolog 1 (C. elegans) | 0.4 | 1.0 | 0.2 | 0.4 |
| Egln3: EGL nine homolog 3 (C. elegans) | 0.7 | 0.6 | 0.4 | 0.4 |
| Siat1: sialyltransferase 1 (beta-galactoside alpha-2,6-sialyltransferase) | 0.6 | 0.4 | 0.4 | 0.3 |
| Stat5b: signal transducer and activator of transcription 5B | 0.7 | 0.4 | 0.4 | 0.9 |

# Candidate Genes

## High-Throughput Functionalisation




Comparative analysis of transcriptional events (physiological / drug-induced apoptotic pathways)

- Extended bioinformatic analysis (annotations, in silico cloning…)
- Systematic reintroduction of candidate genes into model systems (high throughput Gateway™ cloning system, retroviral transduction system)
- Systematic localisation studies (time-lapse video and fluorescent dyes)
- Flexible set-up for “easy to link” experiments, Y2H screening, in vitro assays, recombinant protein production



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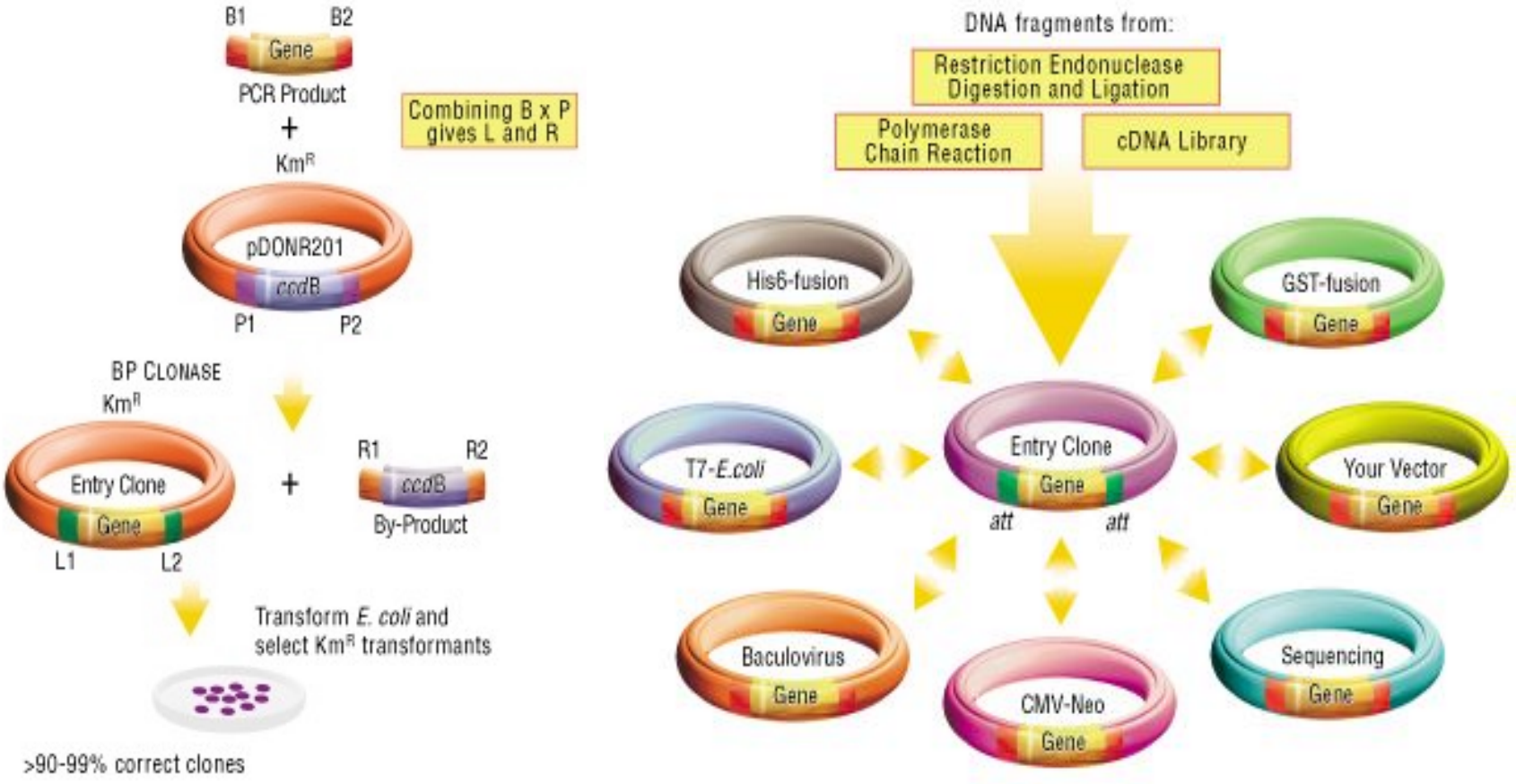
# Candidate Genes

## The Gateway™ System as Core Technology Platform



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# Candidate Genes
## Retroviral Expression Vectors for HT Functionalisation

- N-terminal GFP fusion, constitutive promoter


- IRES GFP, constitutive/inducible promoter


Transfect Phoenix cells

↓

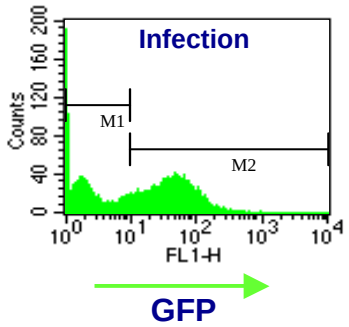
Collect viral supernatant
Infect FL5.12 cells

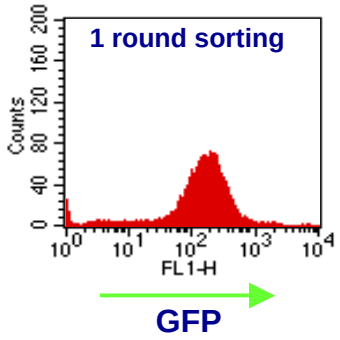
↓

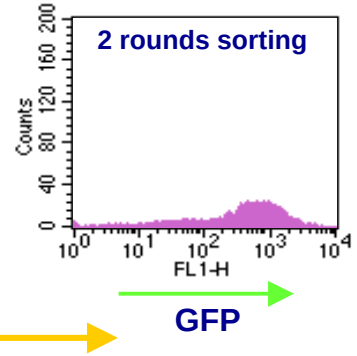
Sort GFP high ⟲ Repeat 2-3x

↓

Apoptosis assay




Sorting rounds →



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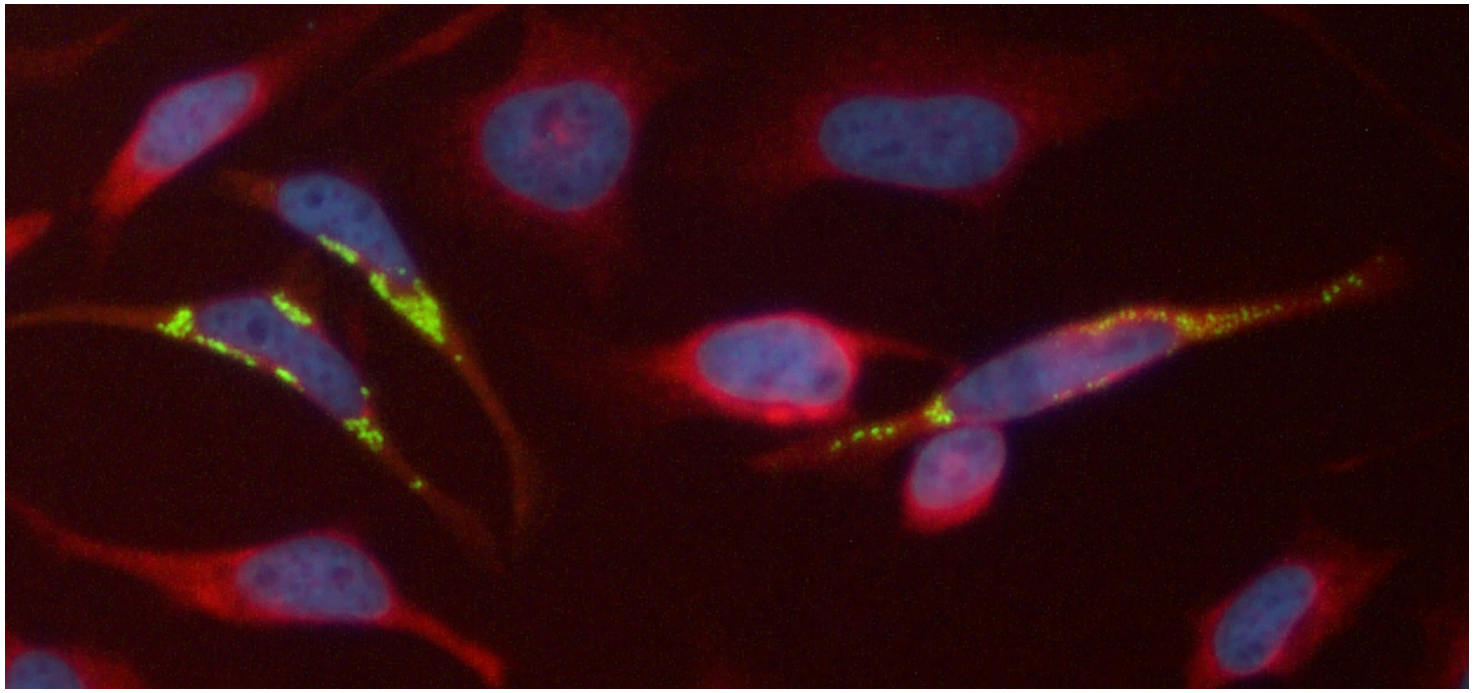
# Candidate Genes

## Ectopic Expression in Various Cellular Systems

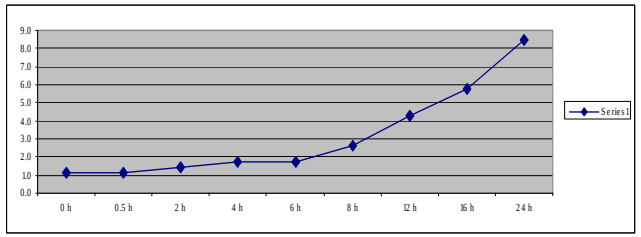


Pro-apoptotic Bax protein (green) during induction of apoptosis
(red: ER, blue: Nuclei)

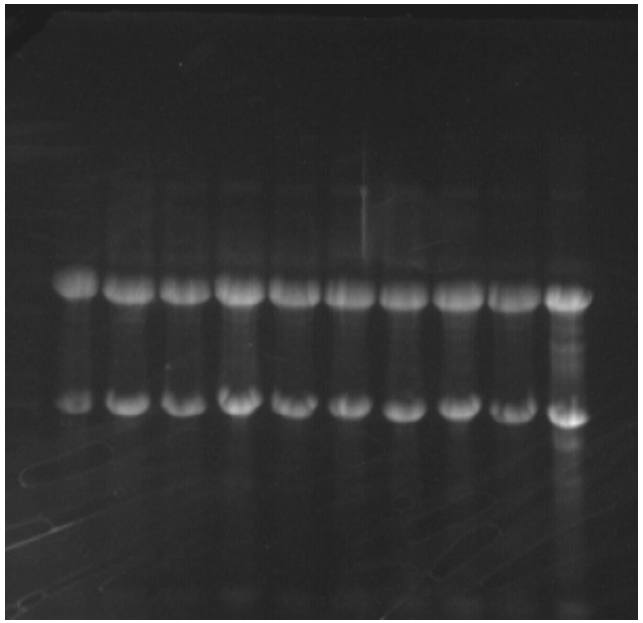
# A Candidate Example

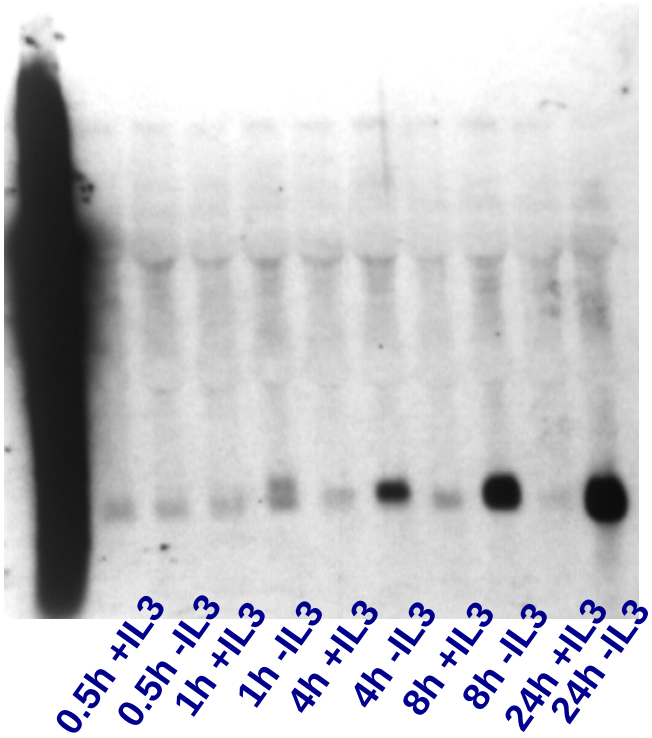
## Hemoglobin α is a Member of "Upregulated Cluster"

**Hemoglobin alpha, adult chain**




**8.5 x**






**Hemoglobin Transcript, 819 bp**



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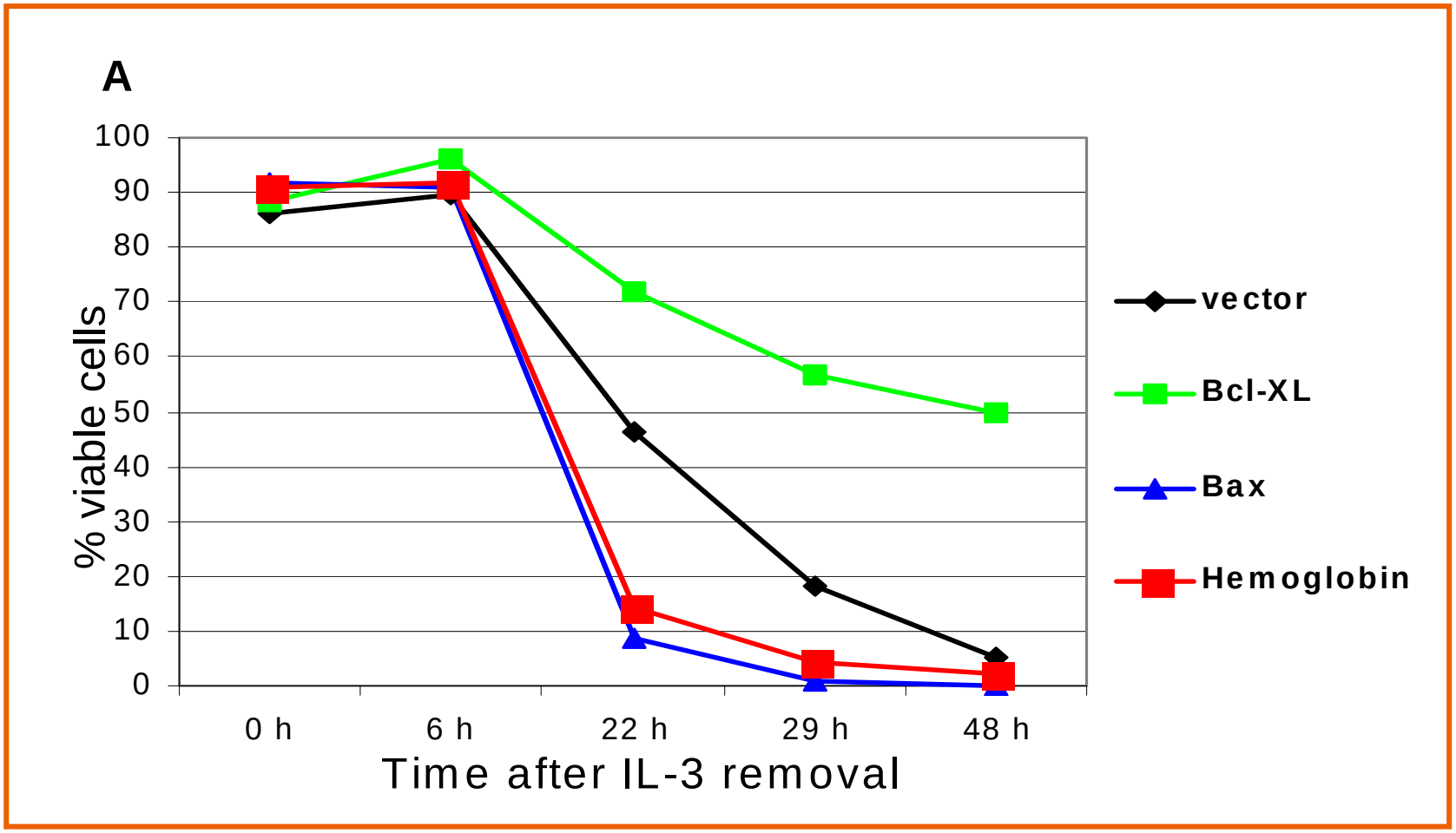
# A Candidate Example

## Hemoglobin α Induces Cell Death as Potently as Bax



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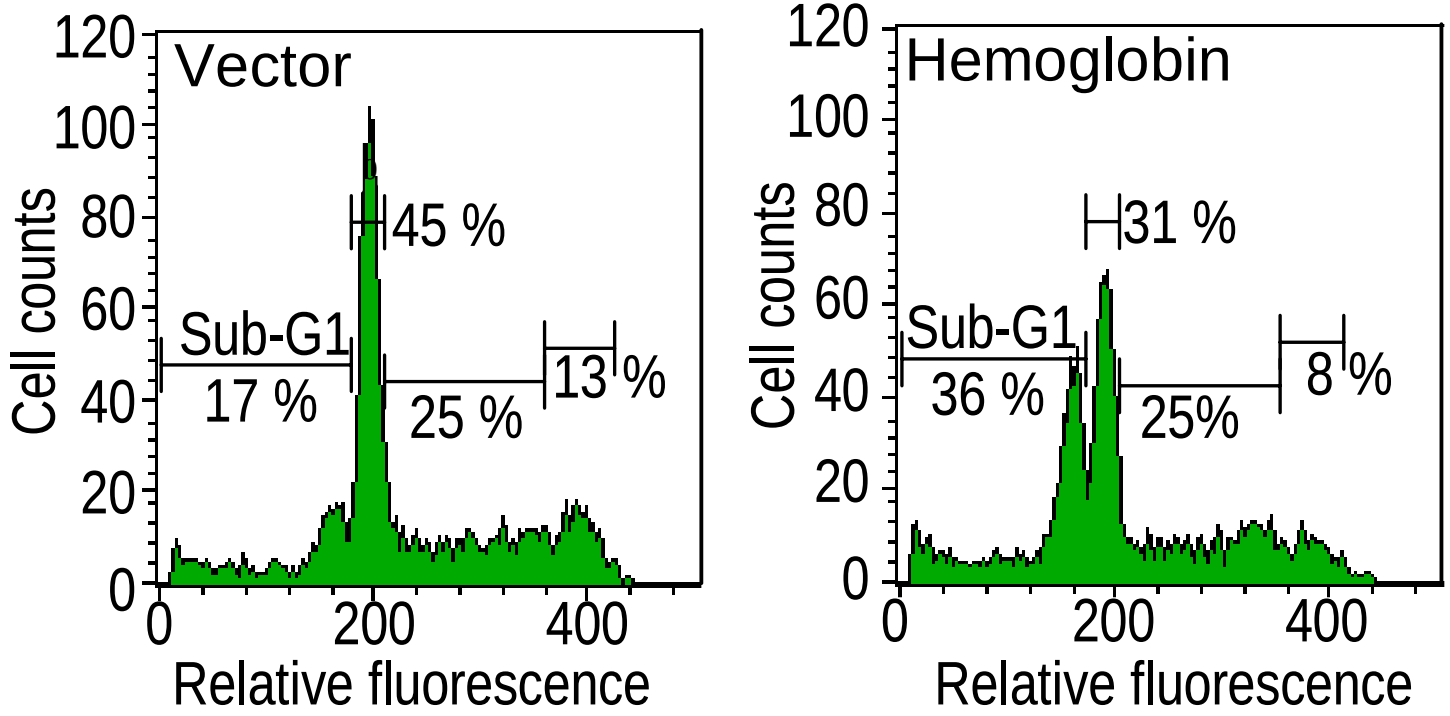
# A Candidate Example
## Hemoglobin α Induces DNA Fragmentation




**Hemoglobin increases the subG1 peak, indicative for apoptosis**



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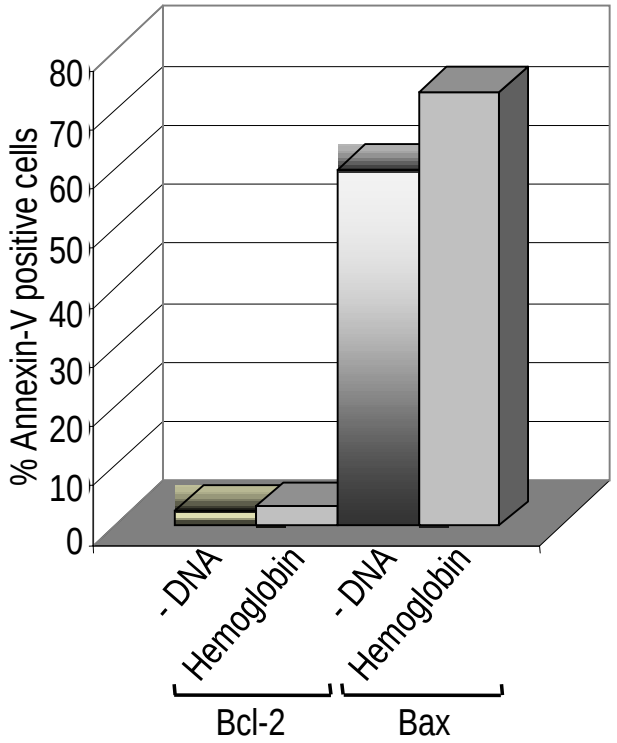
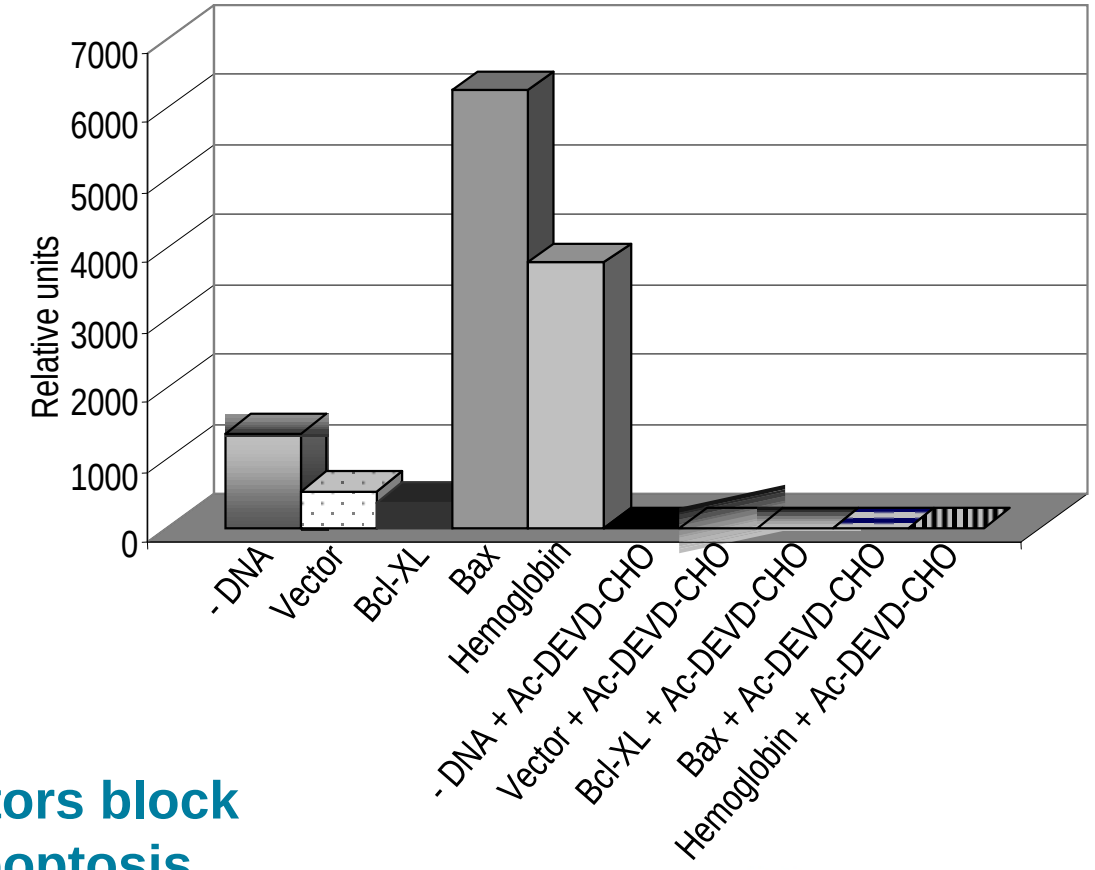
# A Candidate Example

## Hemoglobin α is a New Member of the Central Pathway of Apoptosis in Hematopoietic Cells




**Bcl-2 or caspase inhibitors block hemoglobin induced apoptosis**



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# Summary
## Expression Profiling in Drug Discovery

- Expression profiling routinely used in Pharmaceutical Industry
- Main applications in target discovery and drug profiling
- Sophisticated software tools available to analyse expression data flood
- Clustering algorithms lead to reduction in noise and enrichment in functionality
- Very useful tool to generate biological hypotheses to be tested in functional assays
- Direction of gene expression change not necessarily the same as direction of phenotype

# Thanks to the people doing the work

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**Affymetrix**

**GeneData**

Hans Gmünder

GENE DATA



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