



# IPA robustness analysis

June 15/17, 2020

July 1/3, 2020

*For feedback or questions, please email [bits@vib.be](mailto:bits@vib.be)*

Stephane Plaisance ([stephane.plaisance@vib.be](mailto:stephane.plaisance@vib.be))



Status	Public on Jan 01, 2014
Title	Human Airway Smooth Muscle Transcriptome Changes in Response to Asthma Medications
Organism	<a href="#">Homo sapiens</a>
Experiment type	Expression profiling by high throughput sequencing
Summary	<p><b>Rationale:</b> Asthma is a chronic inflammatory airway disease. The most common medications used for its treatment are <math>\beta</math>2-agonists and glucocorticosteroids, and one of the primary tissues that these drugs target in the treatment of asthma is the airway smooth muscle. We used RNA-Seq to characterize the human airway smooth muscle (HASM) transcriptome at baseline and under three asthma treatment conditions.</p> <p><b>Methods:</b> The Illumina TruSeq assay was used to prepare 75bp paired-end libraries for HASM cells from four white male donors under four treatment conditions: 1) no treatment; 2) treatment with a <math>\beta</math>2-agonist (i.e. Albuterol, 1<math>\mu</math>M for 18h); 3) treatment with a glucocorticosteroid (i.e. Dexamethasone (Dex), 1<math>\mu</math>M for 18h); 4) simultaneous treatment with a <math>\beta</math>2-agonist and glucocorticoid, and the libraries were sequenced with an Illumina Hi-Seq 2000 instrument. The Tuxedo Suite Tools were used to align reads to the hg19 reference genome, assemble transcripts, and perform differential expression analysis using the protocol described in <a href="https://github.com/blancahimes/taffeta">https://github.com/blancahimes/taffeta</a></p>
Overall design	mRNA profiles obtained via RNA-Seq for four primary human airway smooth muscle cell lines that were treated with dexamethasone, albuterol, dexamethasone+albuterol or were left untreated.
Contributor(s)	<a href="#">Himes B, Lu Q</a>
Citation(s)	Himes BE, Jiang X, Wagner P, Hu R et al. RNA-Seq transcriptome profiling identifies CRISPLD2 as a glucocorticoid responsive gene that modulates cytokine function in airway smooth muscle cells. <i>PLoS One</i> 2014;9(6):e99625. PMID: <a href="#">24926665</a>

[https://www.ncbi.nlm.nih.gov/gds/?term=GSE52778\[Accession\]](https://www.ncbi.nlm.nih.gov/gds/?term=GSE52778[Accession])

OPEN ACCESS Freely available online

PLOS ONE



## RNA-Seq Transcriptome Profiling Identifies CRISPLD2 as a Glucocorticoid Responsive Gene that Modulates Cytokine Function in Airway Smooth Muscle Cells

Blanca E. Himes<sup>1,2,3\*</sup>, Xiaofeng Jiang<sup>4\*</sup>, Peter Wagner<sup>4</sup>, Ruoxi Hu<sup>4</sup>, Qiyu Wang<sup>4</sup>, Barbara Klanderman<sup>2</sup>, Reid M. Whitaker<sup>1</sup>, Qingling Duan<sup>1</sup>, Jessica Lasky-Su<sup>1</sup>, Christina Nikolos<sup>5</sup>, William Jester<sup>5</sup>, Martin Johnson<sup>5</sup>, Reynold A. Panettieri Jr.<sup>5</sup>, Kelan G. Tantisira<sup>1</sup>, Scott T. Weiss<sup>1,2</sup>, Quan Lu<sup>4\*</sup>

<sup>1</sup> Channing Division of Network Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts, United States of America, <sup>2</sup> Partners HealthCare Personalized Medicine, Boston, Massachusetts, United States of America, <sup>3</sup> Children's Hospital Informatics Program, Boston, Massachusetts, United States of America, <sup>4</sup> Program in Molecular and Integrative Physiological Sciences, Departments of Environmental Health, and Genetics and Complex Diseases, Harvard School of Public Health, Boston, Massachusetts, United States of America, <sup>5</sup>Pulmonary, Allergy and Critical Care Division, University of Pennsylvania, Philadelphia, Pennsylvania, United States of America

### Abstract

Asthma is a chronic inflammatory respiratory disease that affects over 300 million people worldwide. Glucocorticoids are a mainstay therapy for asthma because they exert anti-inflammatory effects in multiple lung tissues, including the airway smooth muscle (ASM). However, the mechanism by which glucocorticoids suppress inflammation in ASM remains poorly understood. Using RNA-Seq, a high-throughput sequencing method, we characterized transcriptomic changes in four primary human ASM cell lines that were treated with dexamethasone—a potent synthetic glucocorticoid (1  $\mu$ M for 18 hours). Based on a Benjamin-Hochberg corrected p-value <0.05, we identified 316 differentially expressed genes, including both well known (*DUSP1*, *KLF15*, *PER1*, *TSC2D3*) and less investigated (*C7*, *CCDC69*, *CRISPLD2*) glucocorticoid-responsive genes. *CRISPLD2*, which encodes a secreted protein previously implicated in lung development and endotoxin regulation, was found to have SNPs that were moderately associated with inhaled corticosteroid resistance and bronchodilator response among asthma patients in two previously conducted genome-wide association studies. Quantitative RT-PCR and Western blotting showed that dexamethasone treatment significantly increased *CRISPLD2* mRNA and protein expression in ASM cells. *CRISPLD2* expression was also induced by the inflammatory cytokine IL1 $\beta$ , and small interfering RNA-mediated knockdown of *CRISPLD2* further increased IL1 $\beta$ -induced expression of *IL6* and *IL8*. Our findings offer a comprehensive view of the effect of a glucocorticoid on the ASM transcriptome and identify *CRISPLD2* as an asthma pharmacogenetics candidate gene that regulates anti-inflammatory effects of glucocorticoids in the ASM.

**Citation:** Himes BE, Jiang X, Wagner P, Hu R, Wang Q, et al. (2014) RNA-Seq Transcriptome Profiling Identifies CRISPLD2 as a Glucocorticoid Responsive Gene that Modulates Cytokine Function in Airway Smooth Muscle Cells. *PLoS ONE* 9(6): e99625. doi:10.1371/journal.pone.0099625

**Editor:** Jan Peter Tuckermann, University of Ulm, Germany

Received December 6, 2013; Accepted May 17, 2014; Published June 13, 2014

**Copyright:** © 2014 Himes et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

**Funding:** Funding was provided by National Institutes of Health (NIH) U01 HL65899, an NIH Pharmacogenomics Research Network (PGRN) – RIKEN Center for Genomic Medicine (CGM) Global Alliance, R01 HL097796, R01 HL114769, and P30 ES013508. BEH was funded by NIH K99 HL105663. XI was supported by NIH training grant T32 HL007118. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

**Competing interests:** The authors declare that no competing interests exist.

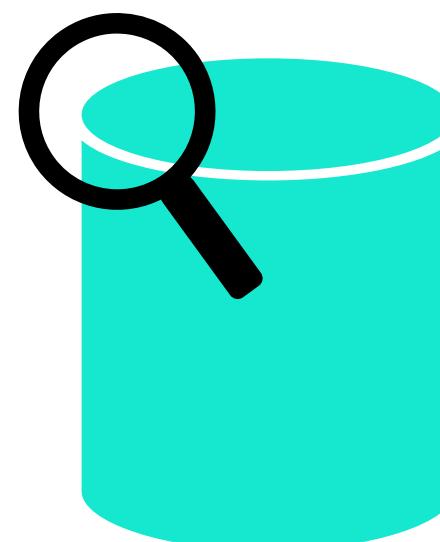
\* Email: blanca.himes@channing.harvard.edu (BH); qiu@hsph.harvard.edu (QJ)

These authors contributed equally to this work.

### Introduction

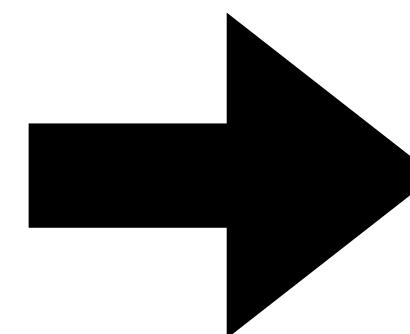
Asthma, a chronic inflammatory respiratory disease that affects over 25 million Americans and 300 million people world-wide, is characterized by variable airflow limitation and airway hyperresponsiveness [1,2]. Glucocorticoids (GCs) are common medications used to treat various inflammatory diseases, including asthma [3]. Inhaled corticosteroids, GC medications that act directly in the lung, are among the most common asthma controller medications and treatment of asthma patients with them leads to improved clinical outcomes, including decreased asthma symptoms and exacerbations [4]. At a cellular level, GCs act by binding to GC receptors (GRs), causing them to translocate to cell nuclei where they modulate transcription of various genes in a tissue-dependent fashion [5]. The anti-inflammatory action of GCs is partly a result of 1) GC-GR complexes stimulating anti-inflammatory genes by directly binding to DNA at glucocorticoid receptor enhancer elements, and of 2) GC-GR complexes inhibiting proinflammatory transcription factors such as nuclear factor kappa-light-chain-enhancer of activated B cells (NFkB) [6]. In addition to directly reducing inflammation, GCs have been shown to affect other asthma-related phenotypes, including bronchodilation [7], airway hyperresponsiveness [8], and airway smooth muscle (ASM) contractility [9].

Many cells and tissues are involved in asthma and are targeted by GCs, including inflammatory [10,11], airway epithelium [12], and ASM [13]. Of these, the ASM is involved in altered airway contractility [14], a major asthma-specific trait that is assessed clinically and for research studies by measures such as bronchodilator response [15] and airway hyperresponsiveness [16].



*Raw mapping counts*

**DESeq2 pipeline**



**DEX**

**Cont**

	ENSGID	baseMean	log2FoldChange	IfcSE	stat	pvalue	padj
1	ENSG00000165995	514.28409	3.3216624	0.13073664	25.407280	2.095444e-142	3.170407e-138
2	ENSG00000152583	985.55928	4.3408121	0.17608576	24.651693	3.529480e-134	2.670051e-130
3	ENSG00000120129	3325.40270	2.8731495	0.11677344	24.604478	1.131211e-133	5.705074e-130
4	ENSG00000101347	13616.93476	3.6065585	0.15175505	23.765657	7.569343e-125	2.863104e-121
5	ENSG00000189221	2294.73000	3.2319825	0.13961723	23.148881	1.491976e-118	4.514719e-115
6	ENSG00000211445	12162.48685	3.5406811	0.15734396	22.502809	3.895921e-112	9.824214e-109
7	ENSG00000162614	5410.96706	1.9889528	0.09230661	21.547241	5.619692e-103	1.214656e-99
8	ENSG00000157214	2925.97934	1.9403761	0.09059393	21.418390	9.004693e-102	1.703013e-98
9	ENSG00000154734	29962.15322	2.2726505	0.11453294	19.842767	1.272588e-87	2.139362e-84
10	ENSG00000179094	763.98622	3.0843241	0.15766714	19.562250	3.244382e-85	4.908750e-82
11	ENSG00000125148	1882.40635	2.0790623	0.10639385	19.541188	4.902803e-85	6.743582e-82
12	ENSG00000163884	545.77230	4.0811465	0.21032420	19.404075	7.129014e-84	8.988498e-81
13	ENSG00000134243	5497.56910	2.1329937	0.11058860	19.287645	6.821066e-83	7.938671e-80
14	ENSG00000139132	1205.02704	2.1776906	0.11309502	19.255406	1.271748e-82	1.374396e-79
15	ENSG00000162493	1082.40622	1.8435294	0.09583897	19.235696	1.860277e-82	1.876399e-79
16	ENSG00000178695	2674.93415	-2.4593228	0.13041743	-18.857317	2.558795e-79	2.419660e-76
17	ENSG00000162692	502.78754	-3.4729419	0.18631326	-18.640336	1.512866e-77	1.346451e-74
18	ENSG00000146250	315.44553	-2.6514559	0.14556181	-18.215326	3.900843e-74	3.278875e-71
19	ENSG00000148848	1348.87125	-1.8136130	0.10181953	-17.812034	5.699996e-71	4.538997e-68
20	ENSG00000198624	2022.68664	2.7847634	0.15667199	17.774481	1.114232e-70	8.429166e-68
21	ENSG00000124766	1319.42200	-2.3315246	0.13494507	-17.277582	6.939659e-67	4.999859e-64

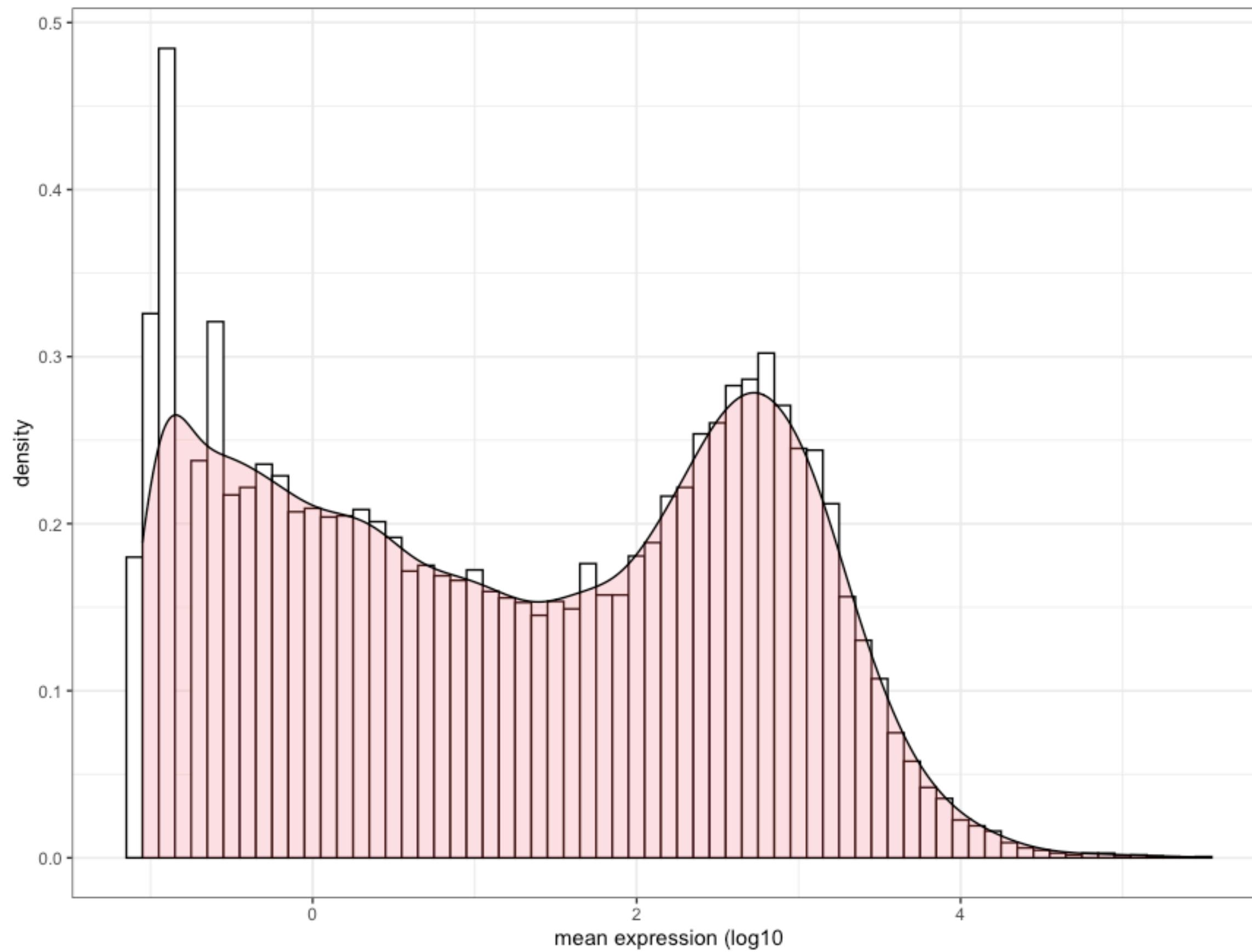


Filter

	ENSGID	baseMean	log2FoldChange	IfcSE	stat	pvalue	padj
1	ENSG00000165995	514.28409	3.3216624	0.13073664	25.407280	2.095444e-142	3.170407e-138
2	ENSG00000152583	985.55928	4.3408121	0.17608576	24.651693	3.529480e-134	2.670051e-130
3	ENSG00000120129	3325.40270	2.8731495	0.11677344	24.604478	1.131211e-133	5.705074e-130
4	ENSG00000101347	13616.93476	3.6065585	0.15175505	23.765657	7.569343e-125	2.863104e-121
5	ENSG00000189221	2294.73000	3.2319825	0.13961723	23.148881	1.491976e-118	4.514719e-115
6	ENSG00000211445	12162.48685	3.5406811	0.15734396	22.502809	3.895921e-112	9.824214e-109
7	ENSG00000162614	5410.96706	1.9889528	0.09230661	21.547241	5.619692e-103	1.214656e-99
8	ENSG00000157214	2925.97934	1.9403761	0.09059393	21.418390	9.004693e-102	1.703013e-98
9	ENSG00000154734	29962.15322	2.2726505	0.11453294	19.842767	1.272588e-87	2.139362e-84
10	ENSG00000179094	763.98622	3.0843241	0.15766714	19.562250	3.244382e-85	4.908750e-82
11	ENSG00000125148	1882.40635	2.0790623	0.10639385	19.541188	4.902803e-85	6.743582e-82
12	ENSG00000163884	545.77230	4.0811465	0.21032420	19.404075	7.129014e-84	8.988498e-81
13	ENSG00000134243	5497.56910	2.1329937	0.11058860	19.287645	6.821066e-83	7.938671e-80
14	ENSG00000139132	1205.02704	2.1776906	0.11309502	19.255406	1.271748e-82	1.374396e-79
15	ENSG00000162493	1082.40622	1.8435294	0.09583897	19.235696	1.860277e-82	1.876399e-79
16	ENSG00000178695	2674.93415	-2.4593228	0.13041743	-18.857317	2.558795e-79	2.419660e-76
17	ENSG00000162692	502.78754	-3.4729419	0.18631326	-18.640336	1.512866e-77	1.346451e-74
18	ENSG00000146250	315.44553	-2.6514559	0.14556181	-18.215326	3.900843e-74	3.278875e-71
19	ENSG00000148848	1348.87125	-1.8136130	0.10181953	-17.812034	5.699996e-71	4.538997e-68
20	ENSG00000198624	2022.68664	2.7847634	0.15667199	17.774481	1.114232e-70	8.429166e-68
21	ENSG00000124766	1319.42200	-2.3315246	0.13494507	-17.277582	6.939659e-67	4.999859e-64

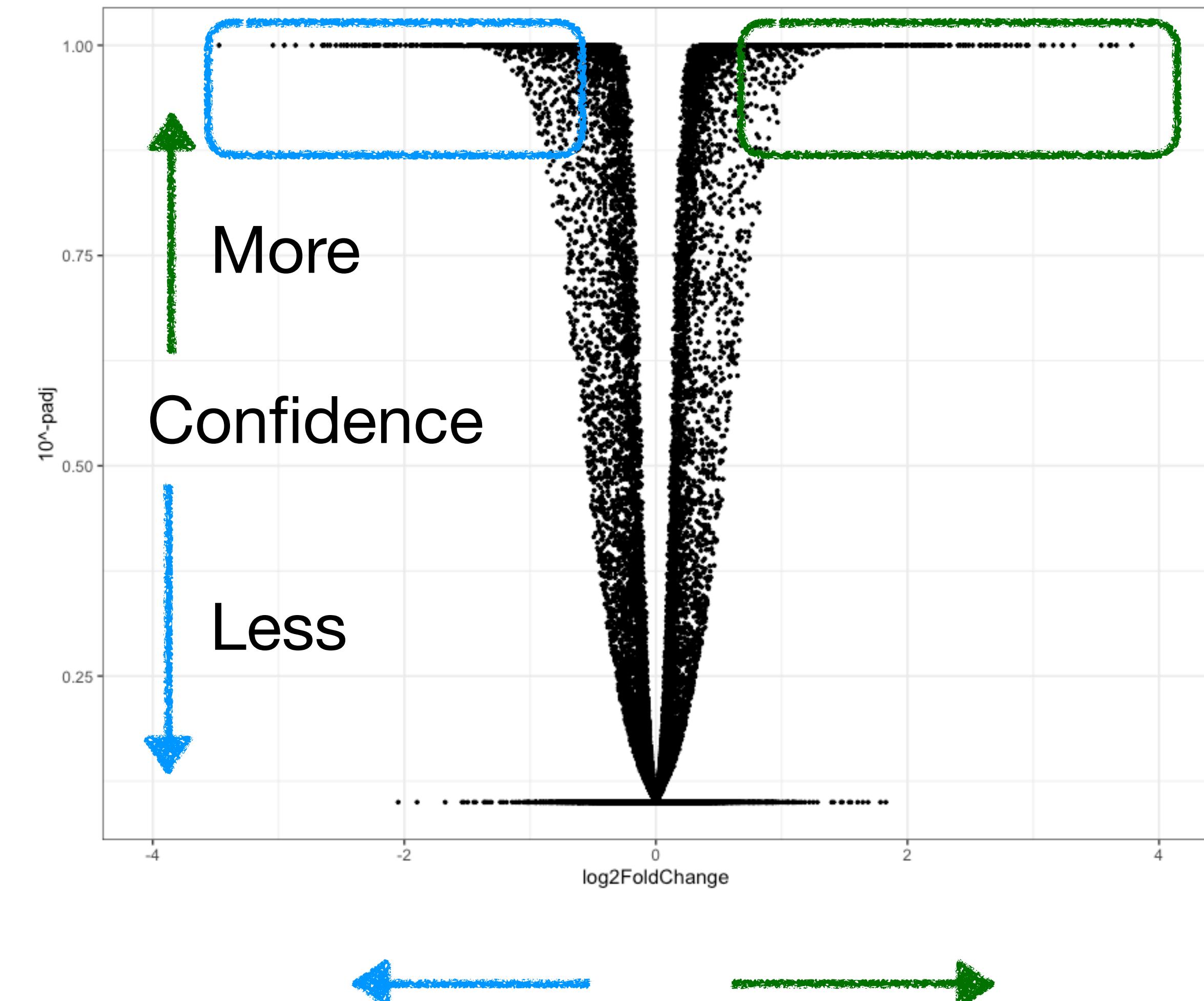
quick exploration of our dataset

## Distribution of expression levels 'across all samples'



Low ← expression level → High

## Differential expression Dex / Cont

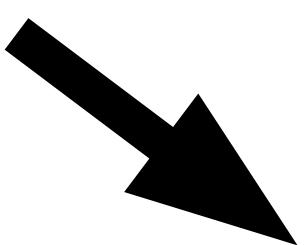
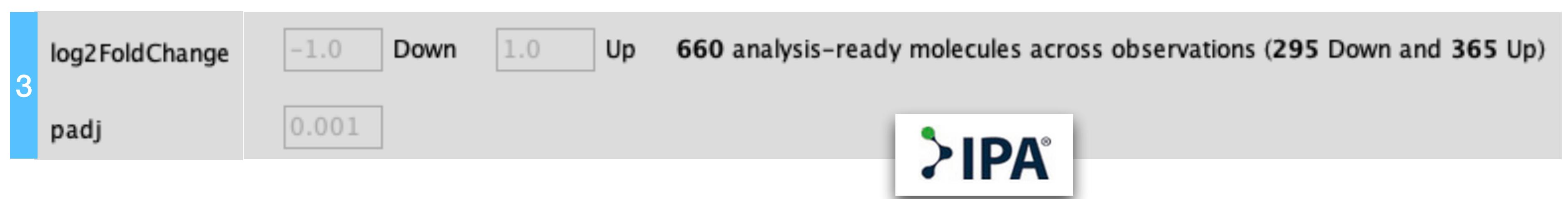
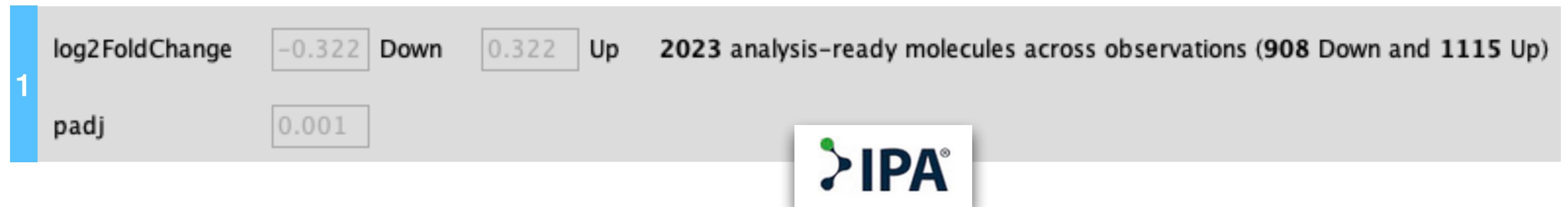


Down → Up

# IPA robustness analyses

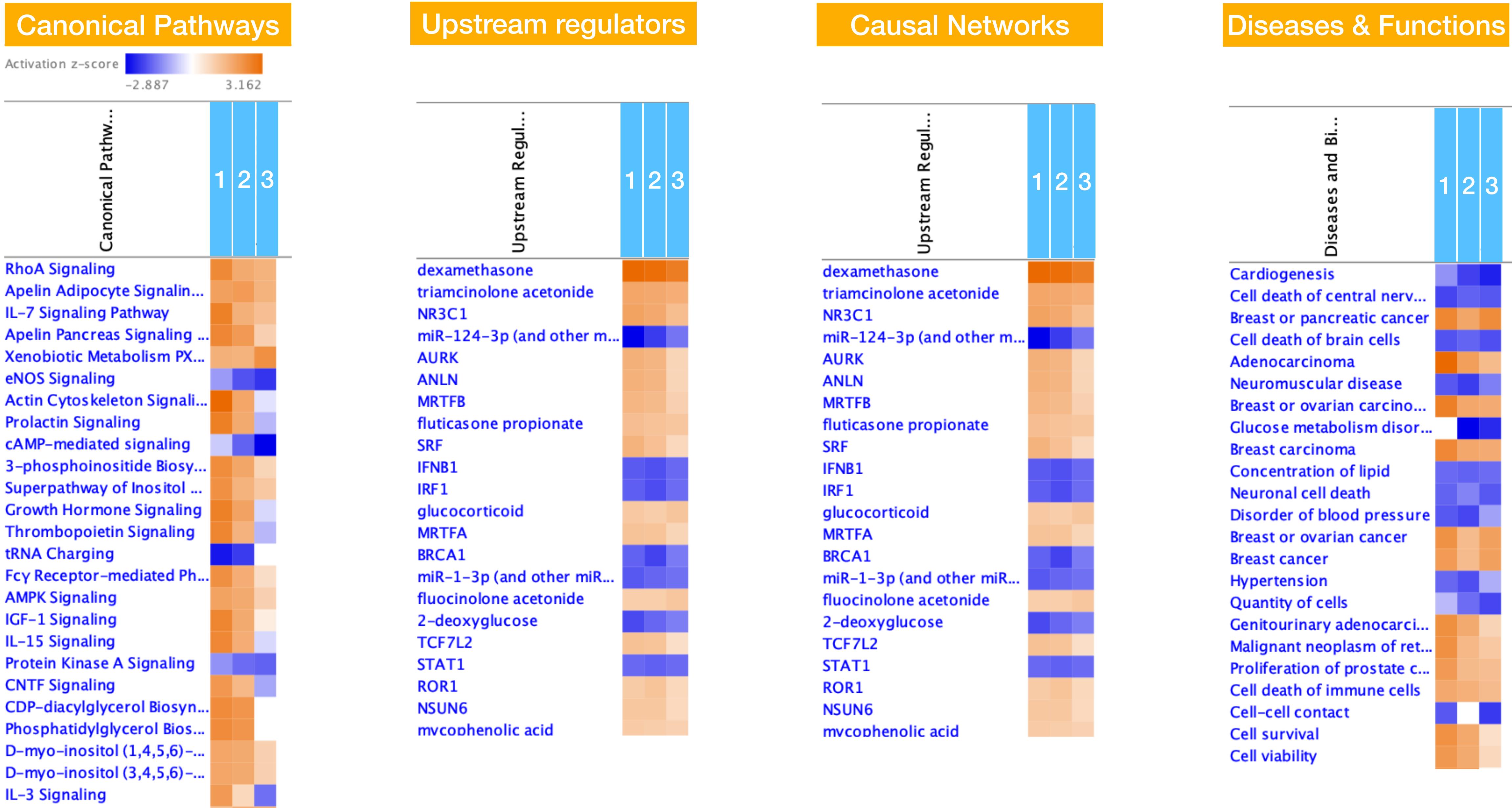
- applying different cutoffs to DE data
- data acquired with a low sensitivity technology
- data acquired with a low coverage technology
- Importance of the background

# DE cutoff effect



Compare

*all features are quite robust to cutoff but harder cutoff would harm\**

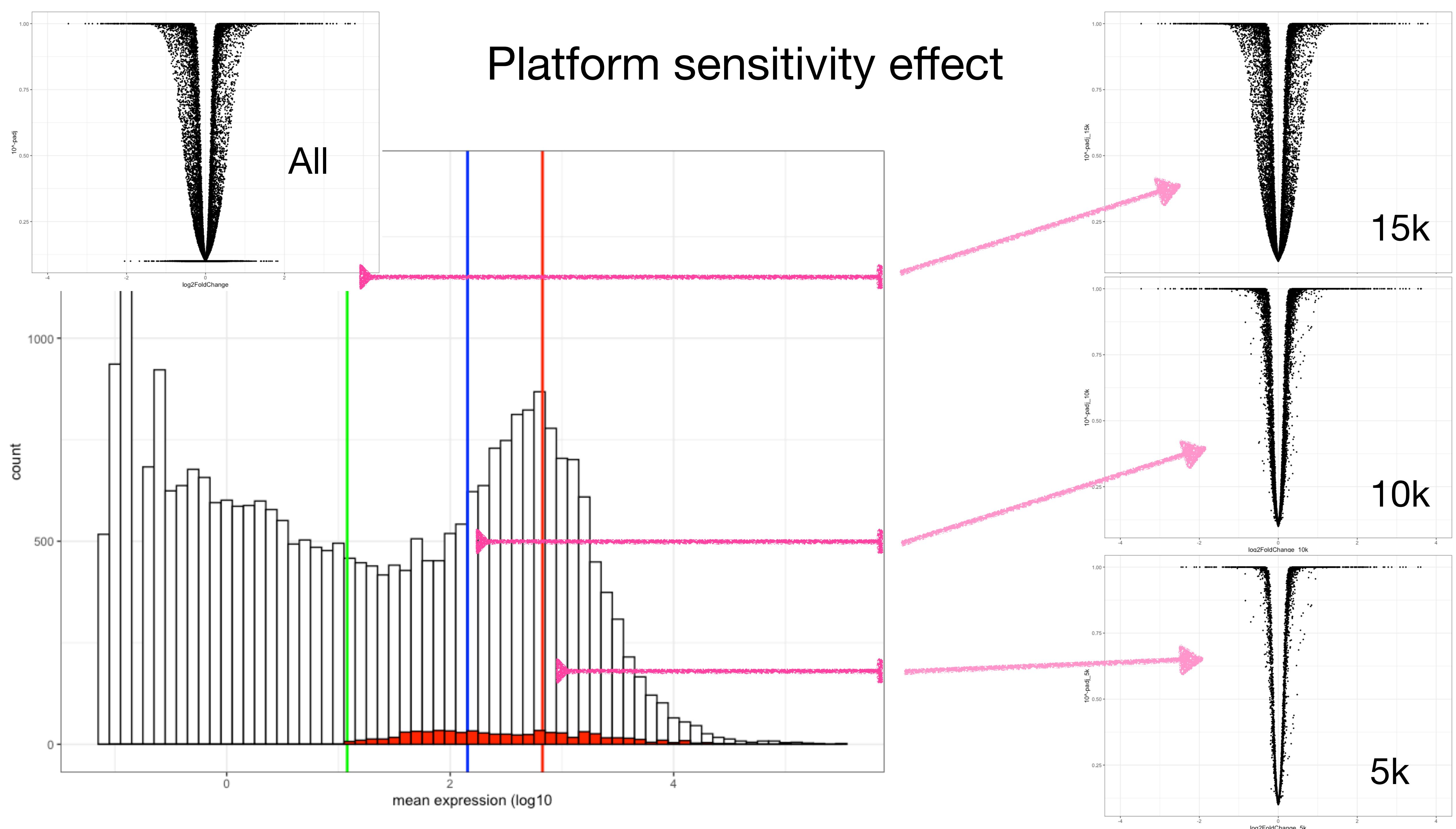


\*not shown

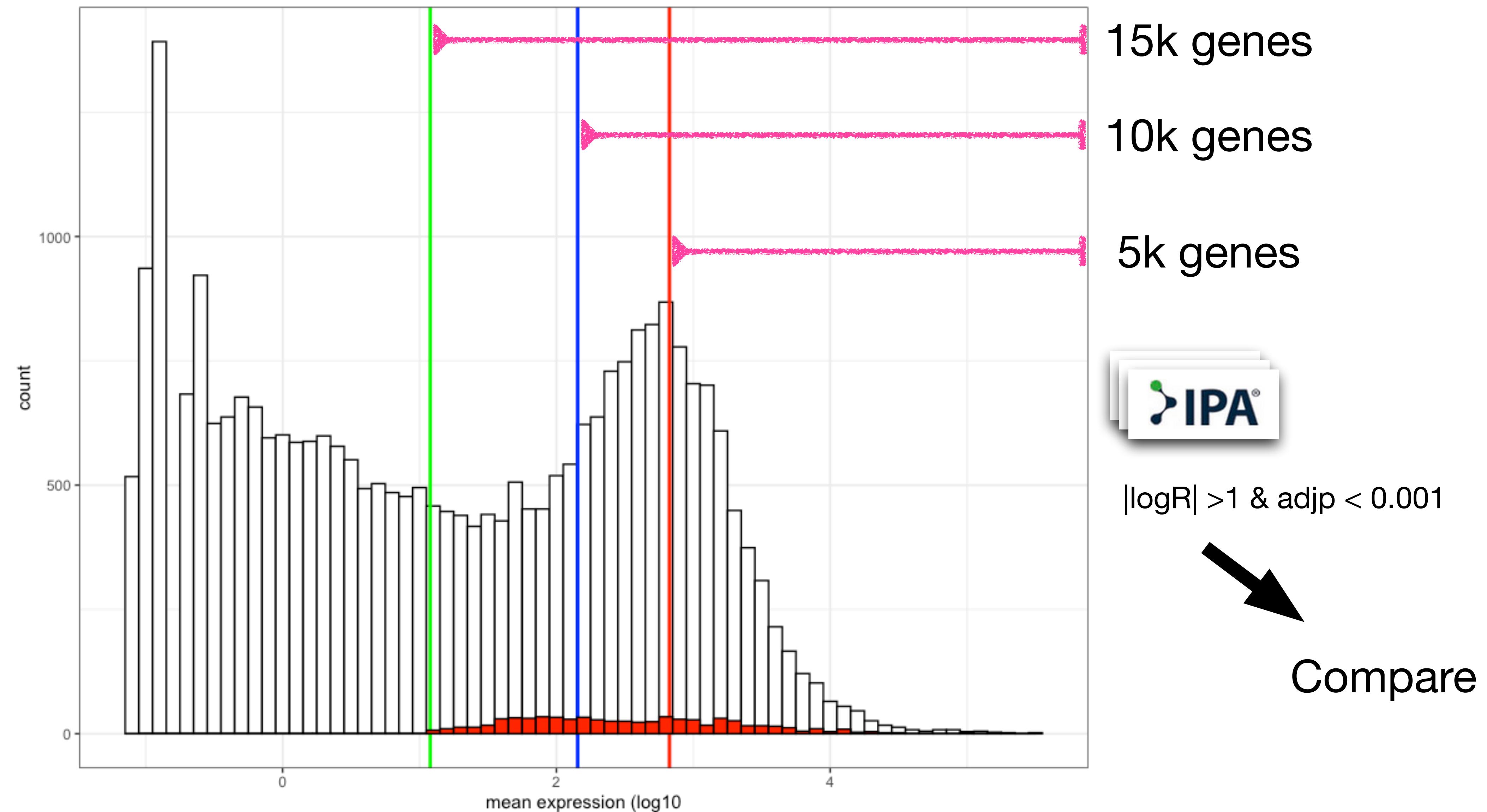
# IPA robustness analyses

- applying different cutoffs to DE data
- data acquired with a low sensitivity technology
- data acquired with a low coverage technology
- Importance of the background

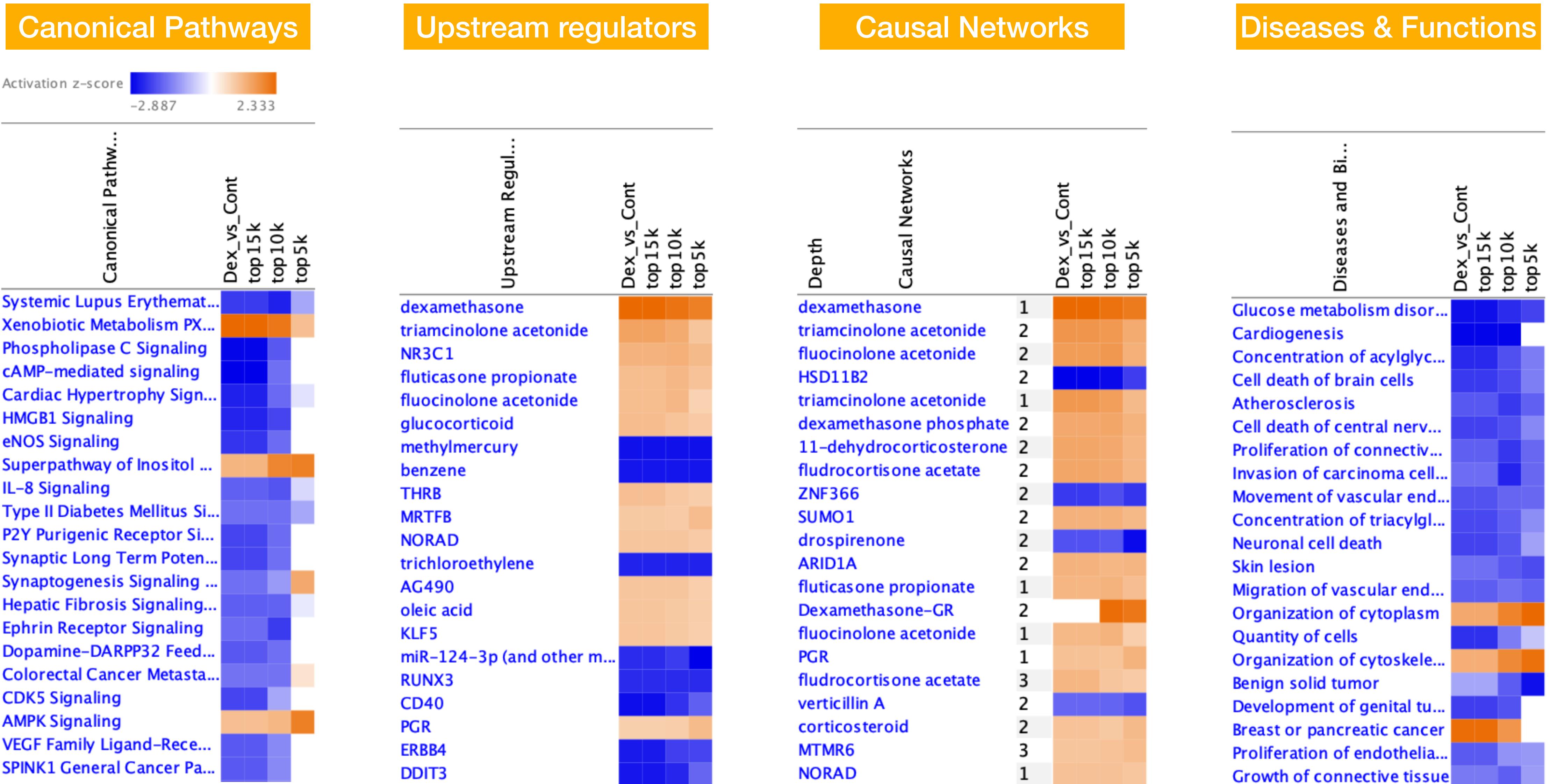
# Platform sensitivity effect



# robustness to sensibility of detection



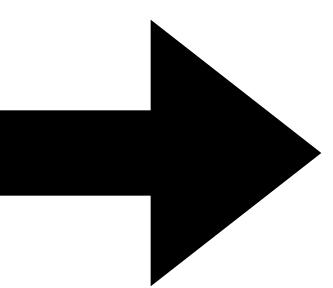
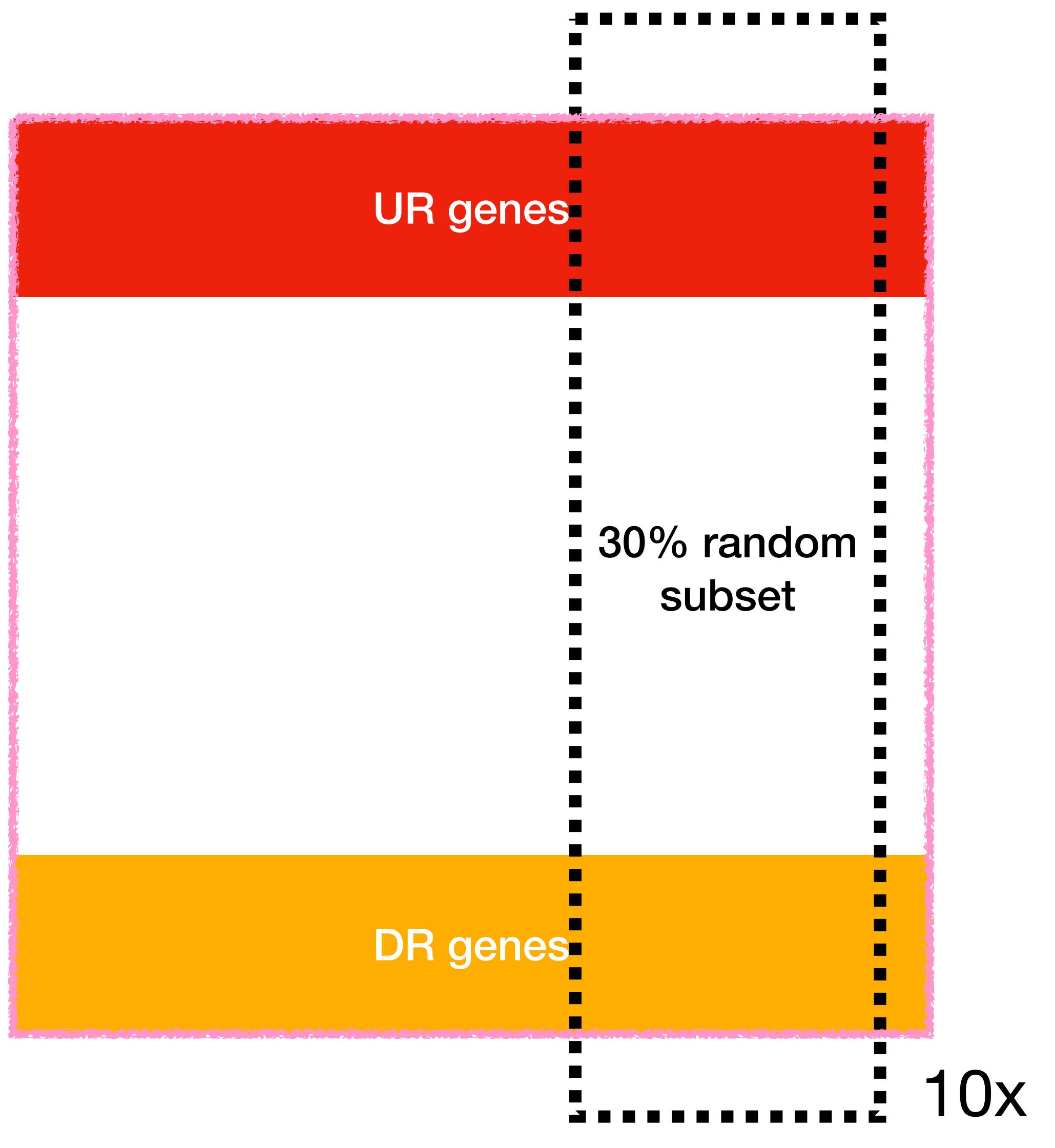
*large data loss leads to less sensitivity due to loss of lower-expression DE genes*



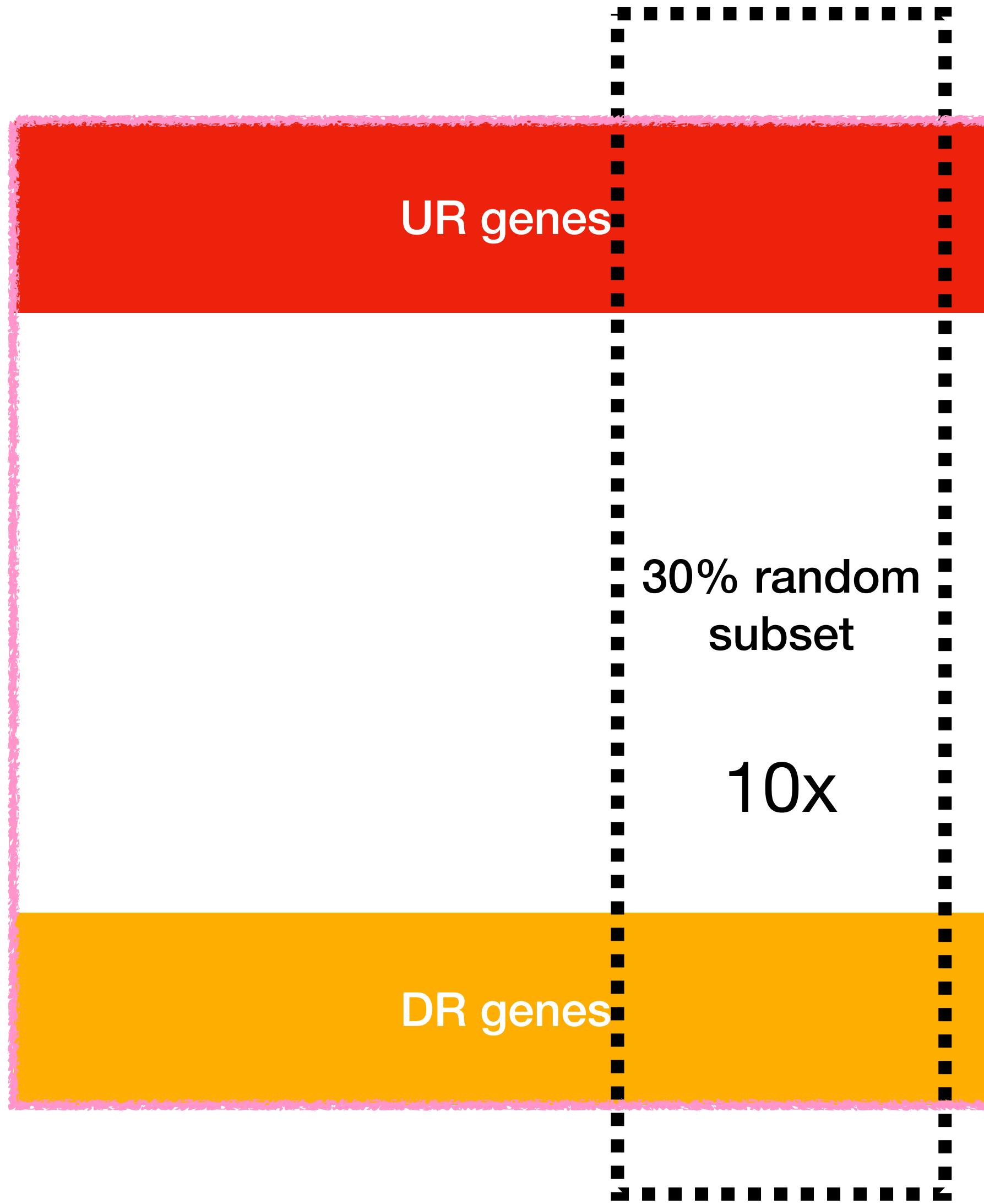
# IPA robustness analyses

- applying different cutoffs to DE data
- data acquired with a low sensitivity technology
- **data acquired with a low coverage technology**
- Importance of the background

# Sampling effect



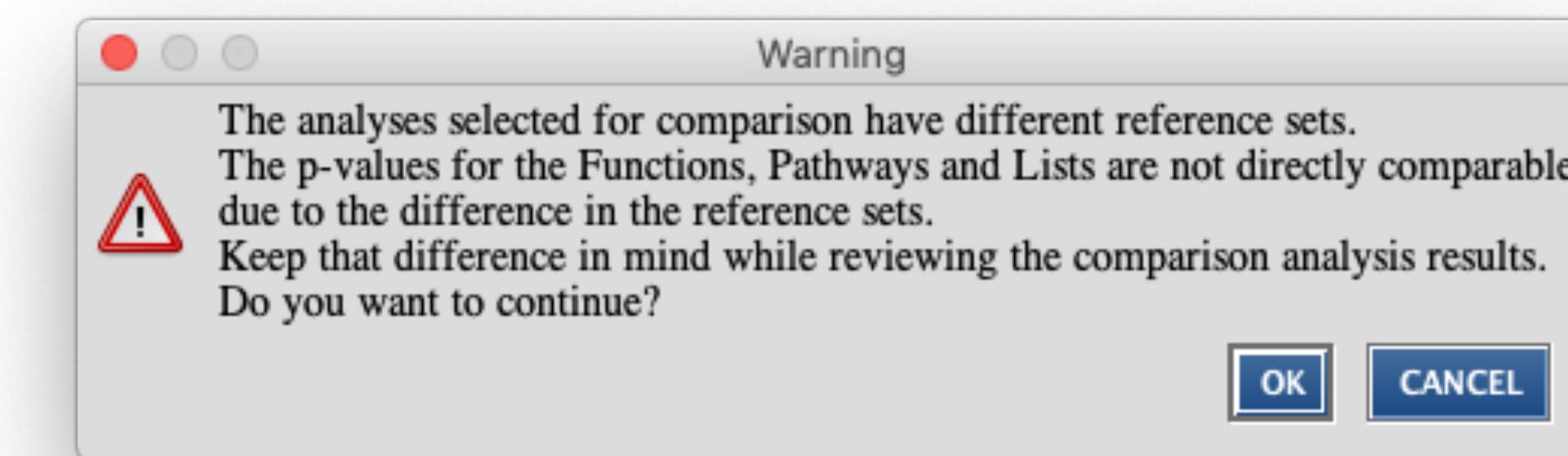
# Robustness to random sampling



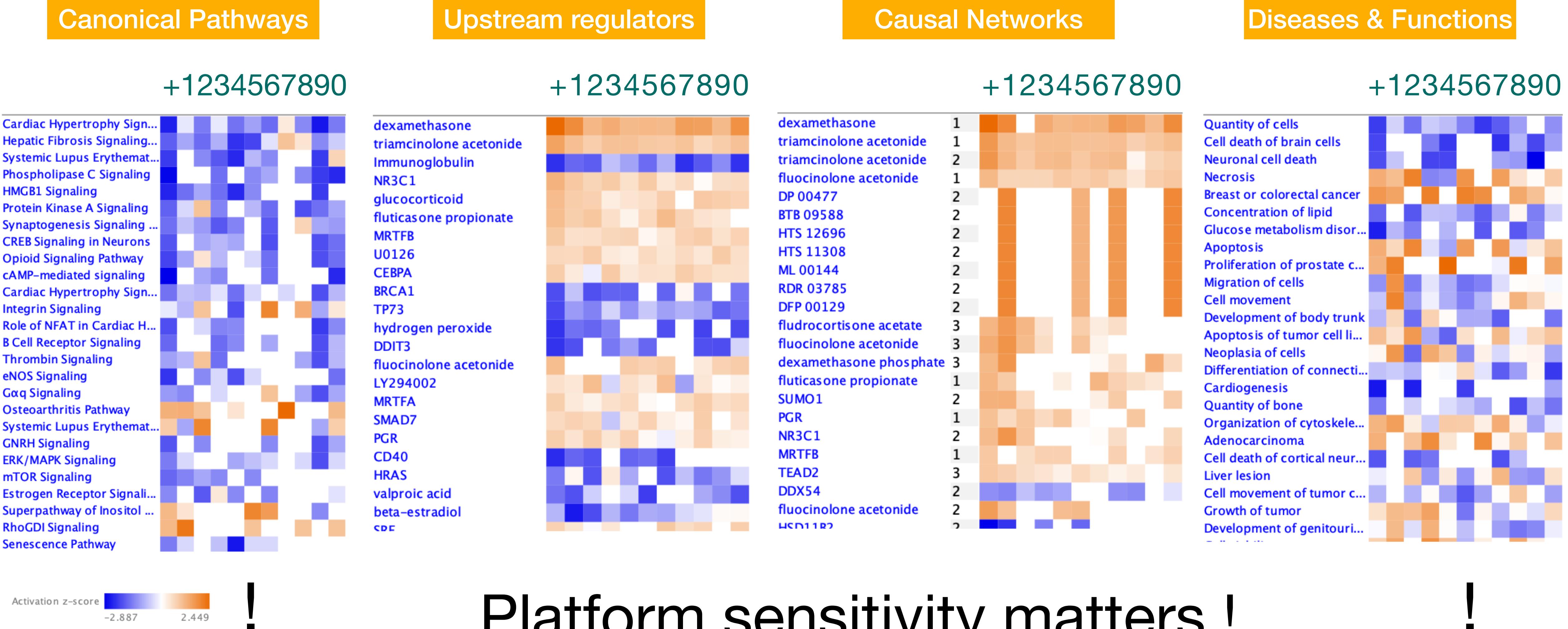
10x core analysis  
KB as background

$|logR| > 1$  &  $adjp < 0.001$

Compare



*Platforms that only ‘see’ a fraction of the truth are less robust although globally IPA resists well to random sampling*

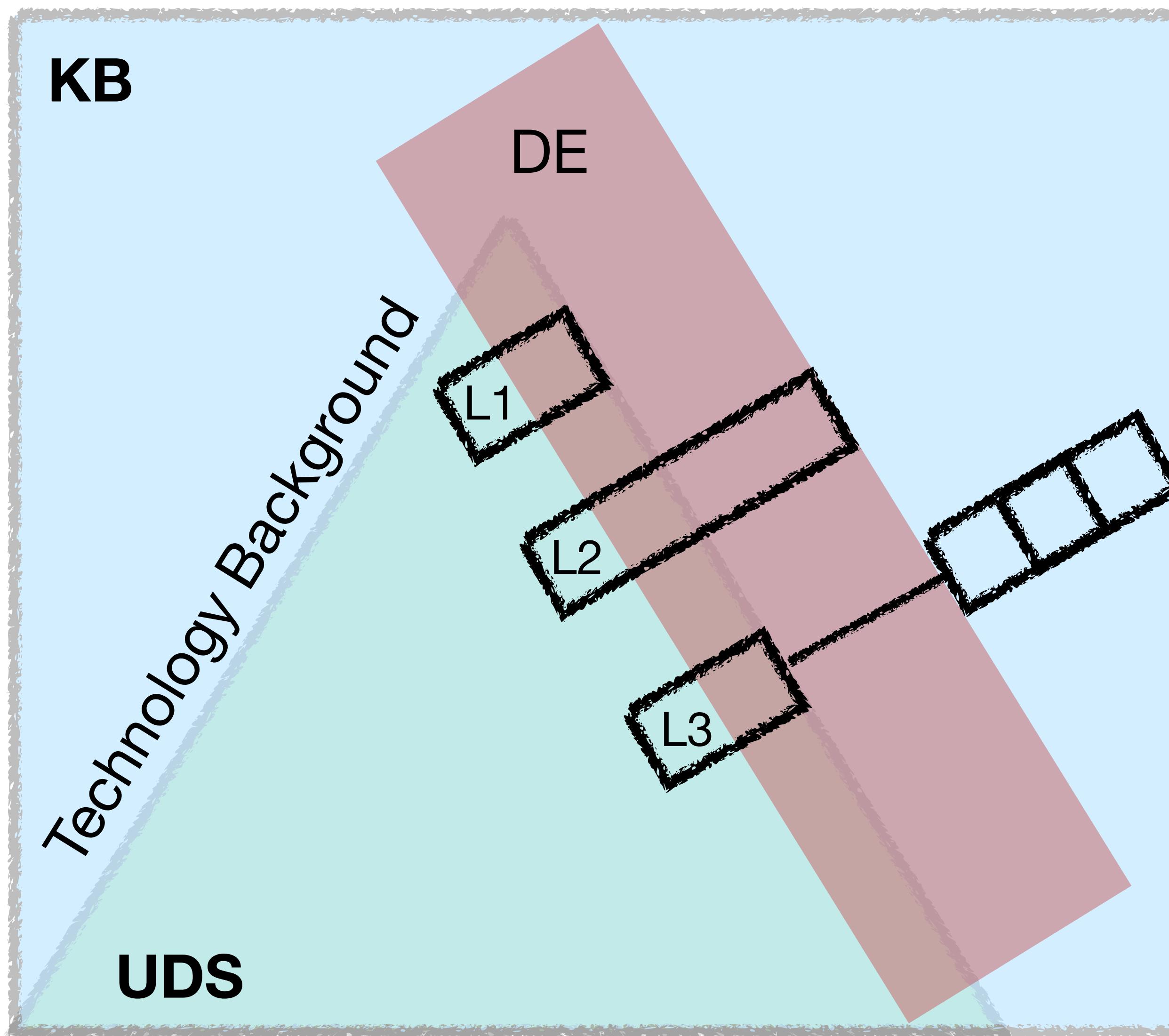


# IPA robustness analyses

- applying different cutoffs to DE data
- data acquired with a low sensitivity technology
- data acquired with a low coverage technology
- Importance of the background

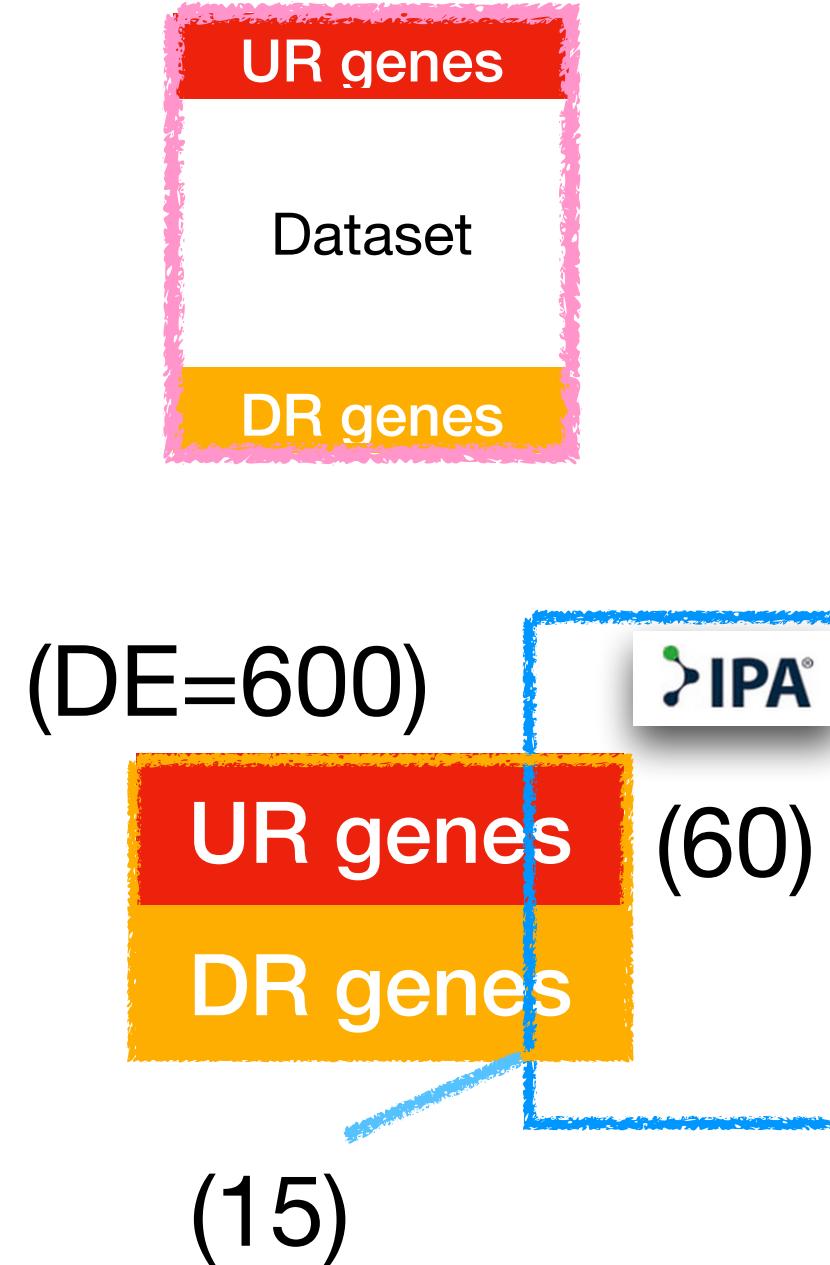
# Background effect

## Genome Background

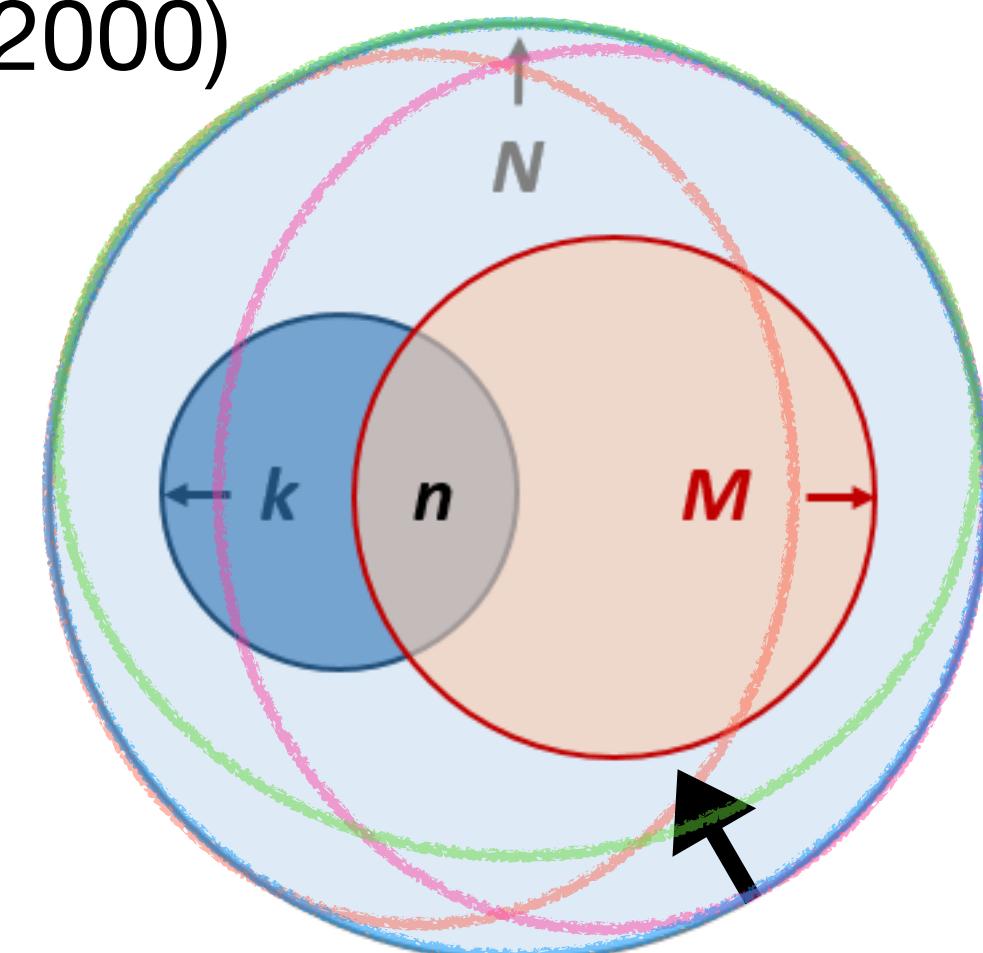


	UDS	KB	Effect
L1	50%	50%	=
L2	50%	75%	<i>underestimate</i>
L3	50%	20%	<i>overestimate</i>

# Simulation of a background & sampling effects



N (22000)



Part of the background is missed

k=600, n=15, M=60	
Background	Enrich test*
IPA KB	3.671516e-12
15k	9.821754e-10
10k	2.739608e-07
5k	0.001280419

only 5 out of 15 DE are quantified

k=600, n=5, M=60	
Background	Enrich test
IPA KB	0.005716096
15k	0.03180919
10k	0.1469838
5k	0.7344489

*under-estimate*

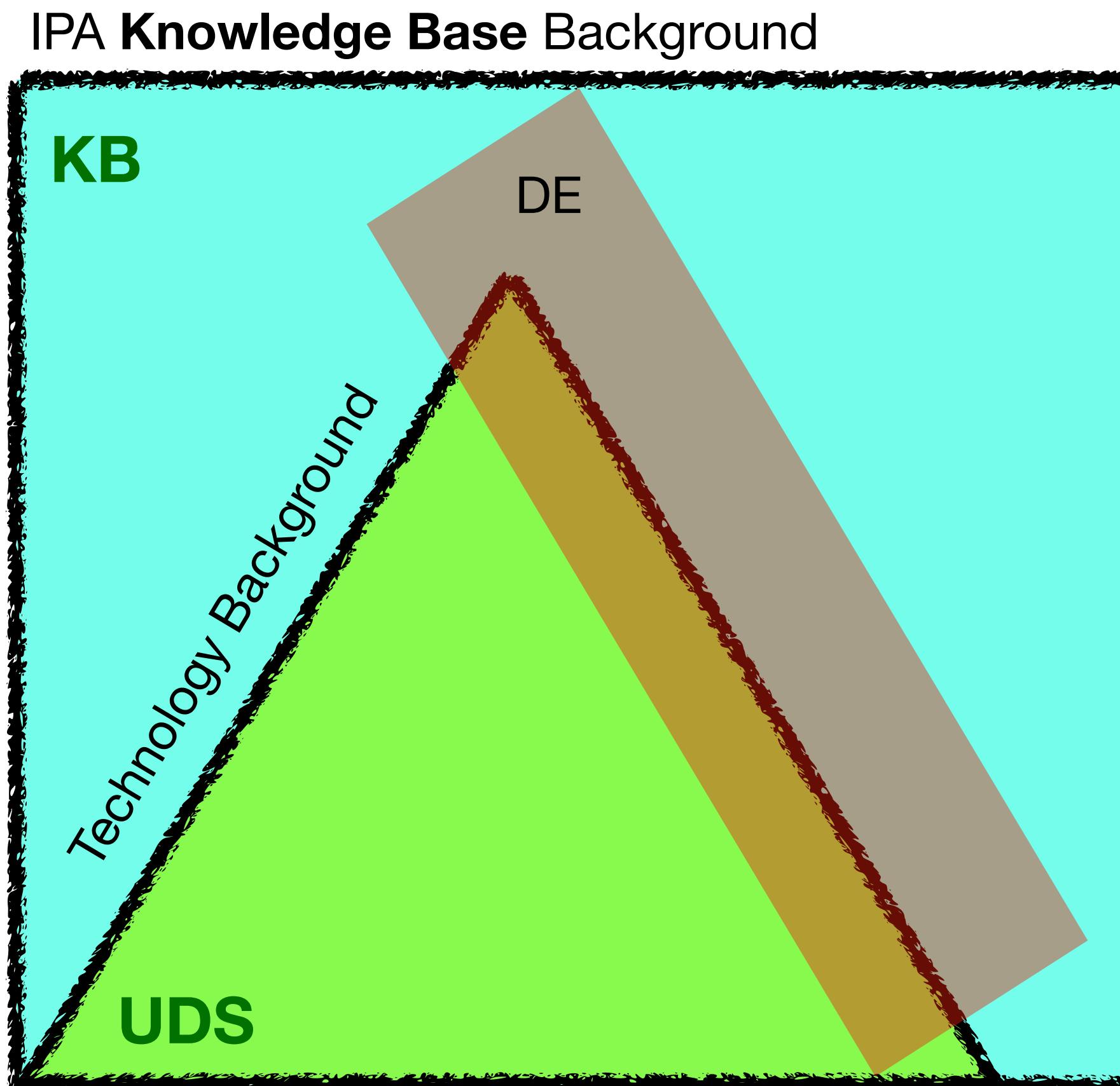
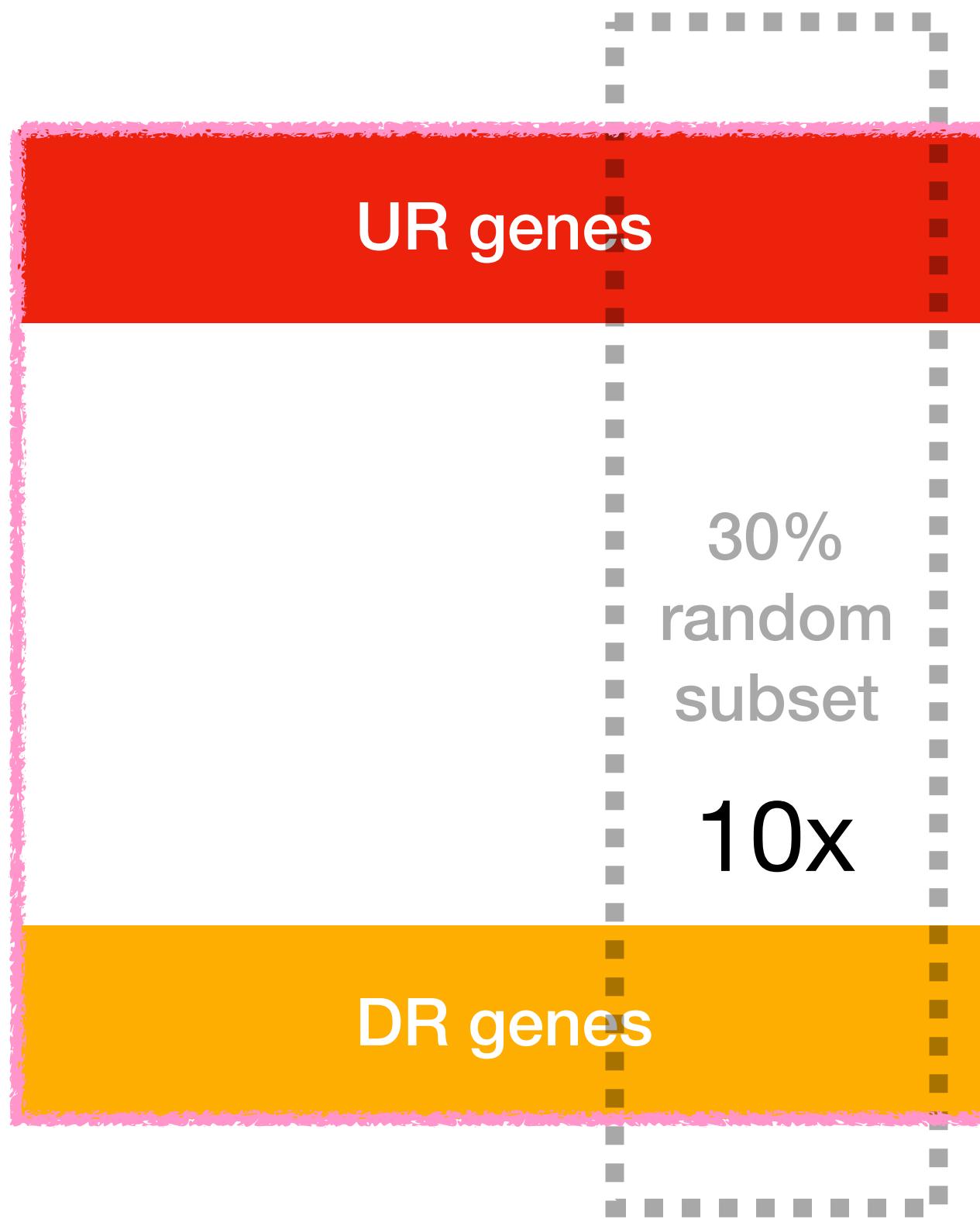
20 out of 60 from list are quantified

k=600, n=15, M=20	
Background	Enrich test
IPA KB	3.358551e-22
15k	1.468597e-19
10k	8.944017e-17
5k	4.627636e-12

*over-estimate*

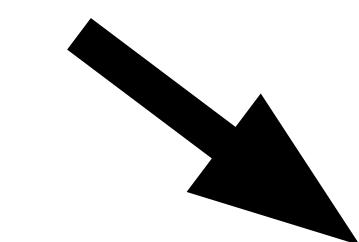
\*  $p_{hyper}(q, m, n, k, lower.tail = FALSE, log.p = FALSE)$

# Sampling x Background effect

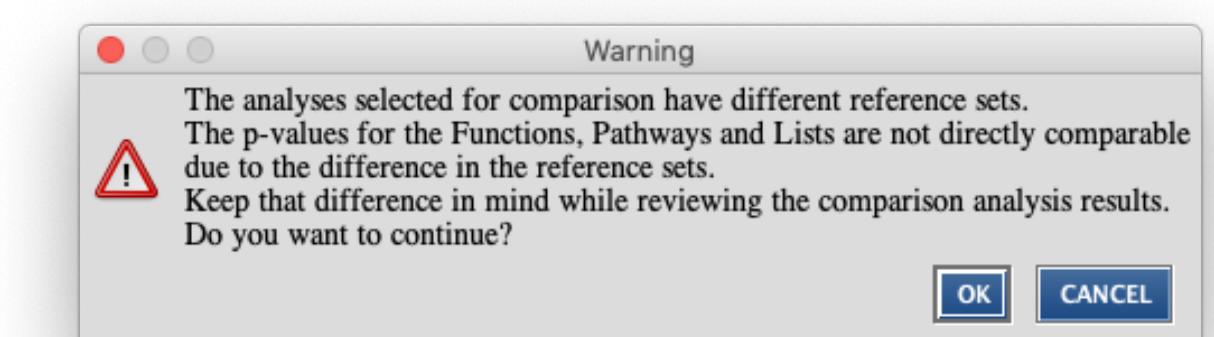


2x 10x core analysis  
different backgrounds

$|logR| > 1$  &  $adjp < 0.001$

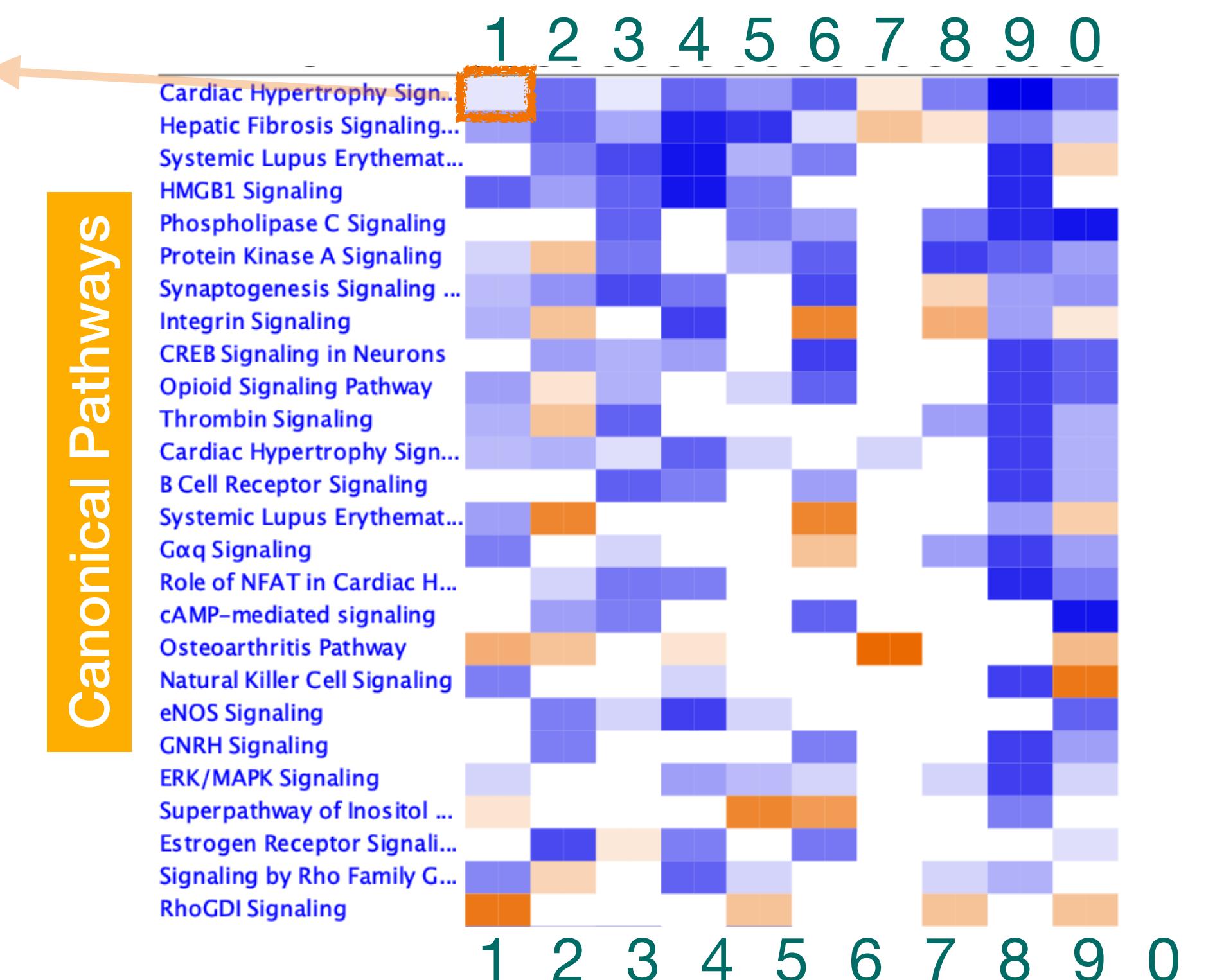


Compare

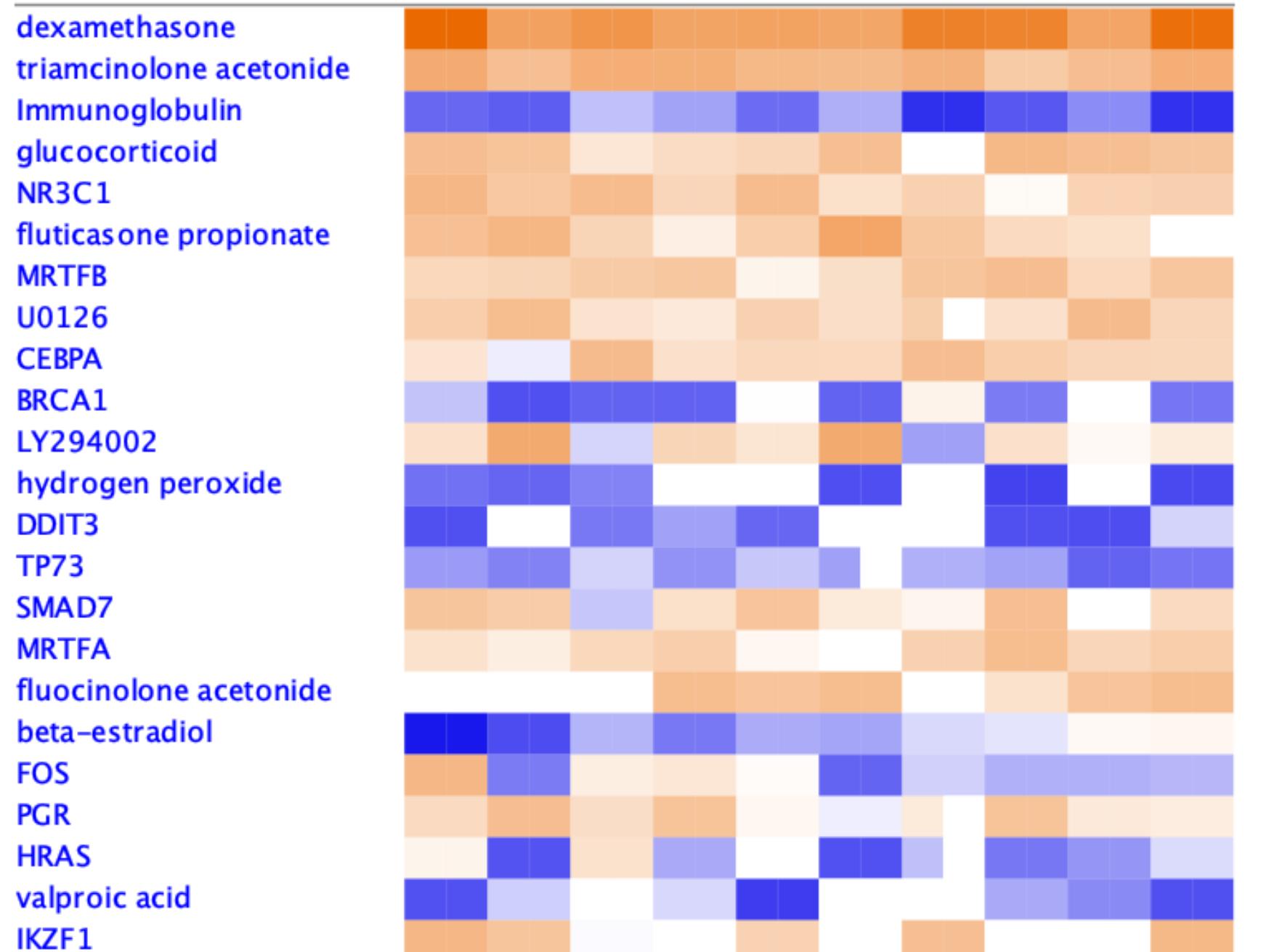


**KB** | **UD**

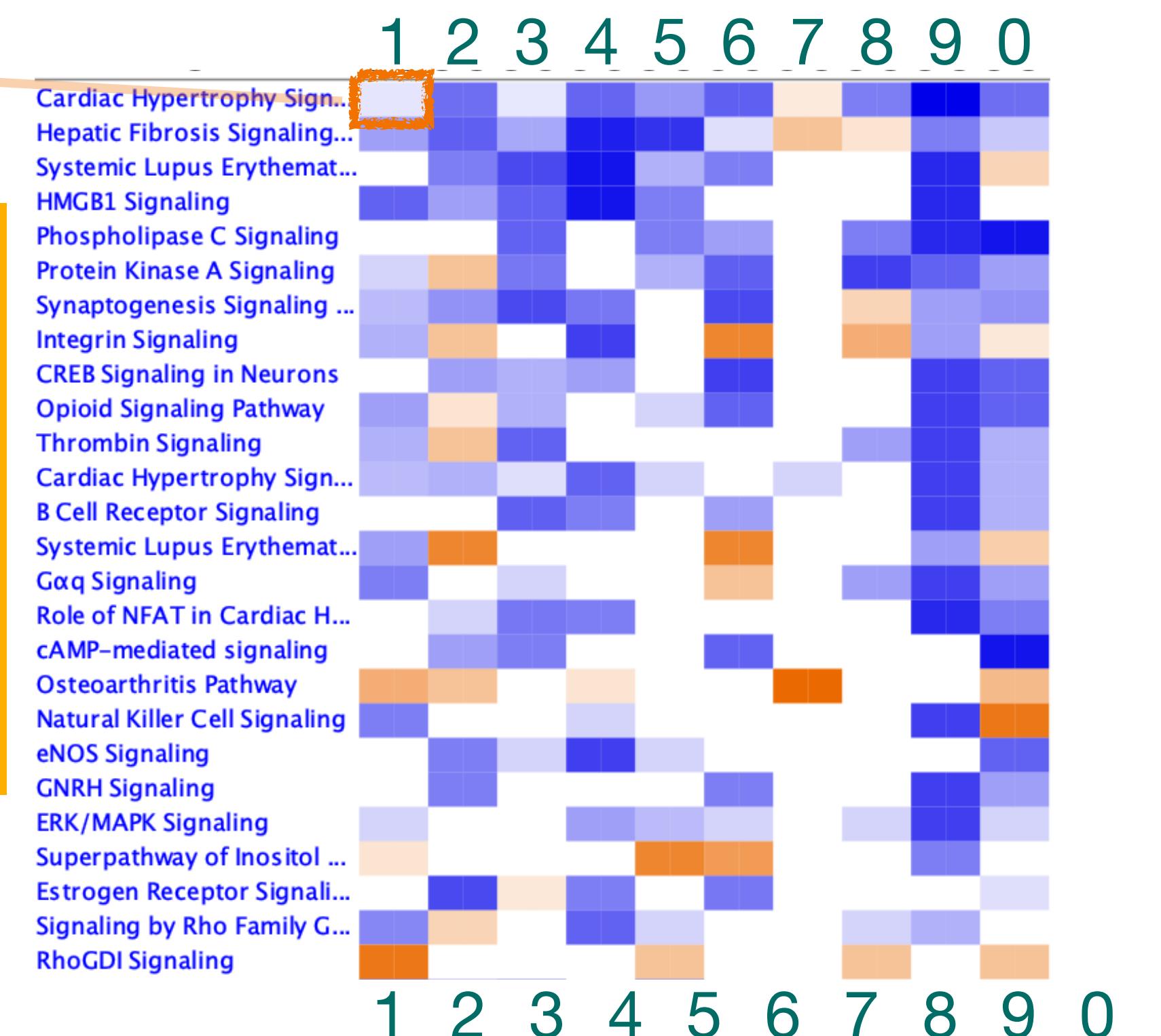
S



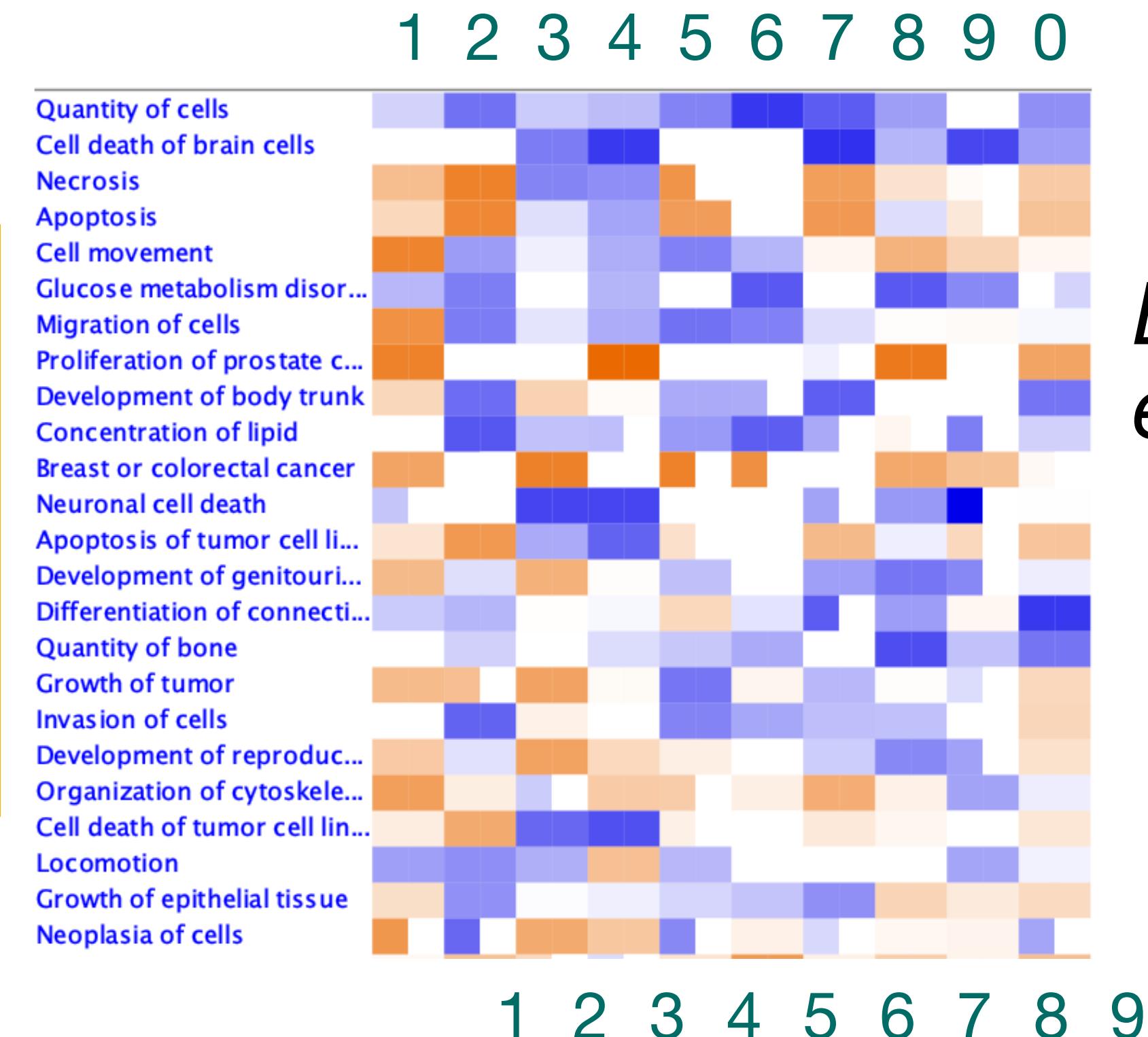
## Upstream regulators



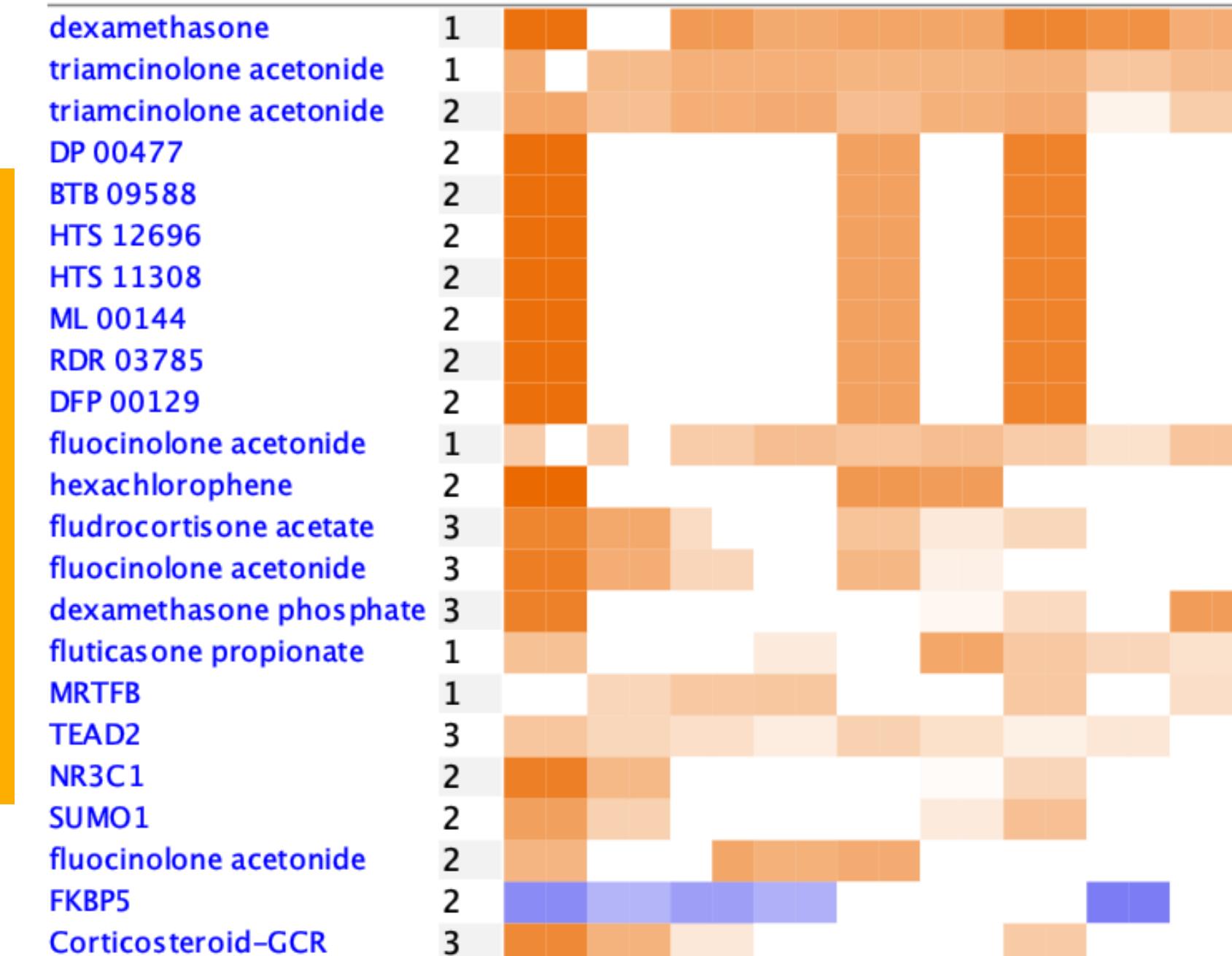
# Canonical Pathways



# Diseases & Functions



# Causal Networks



# *Background effect*

!

IPA

<https://www.bits.vib.be/software-overview/ingenuity-pathways-analysis>

bits.vib.be/software-overview/ingenuity-pathways-analysis

Extensions WA Home 365 SMRT ONT R710 NC VIB BITS Covid-19 perso myPages Git Biocomputing LOCALHOST utils

VIB Bioinformatics Core

Search

HOME TRAINING SOFTWARE SUPPORT INFORMATICS SERVICES BIOIT@VIB ABOUT US

Ingenuity Pathways Analysis

 With Ingenuity Pathways Analysis you can summarize a variety of biological information around your gene of interest. Create visually appealing networks of your pathway or your gene lists and start discovering biology! Which biological processes does your gene list represent? IPA can tell you immediately, based on their curated information source extracted from the literature.  
We have several free IPA licenses for use by VIB scientists.

Get it!

How to access IPA? (VIB scientists only)

IPA news

Read the latest release notes ([here](#)).  
Read the archived release notes ([here](#)).

Online Tutorials

Recorded IPA webinars and tutorial videos (in particular the 3-part series covering most of IPA)

Software Support

Overview

- > CLC Main Workbench
- > Tibco Spotfire
- > CLC Genomics Workbench
- > ELN
- > Genevestigator
- > GraphPad Prism
- > IPA
- > MATLAB
- > OpenRefine
- > qbase+
- > SnapGene
- > Galaxy
- > Developed@VIB
- > FlowJo

Software News