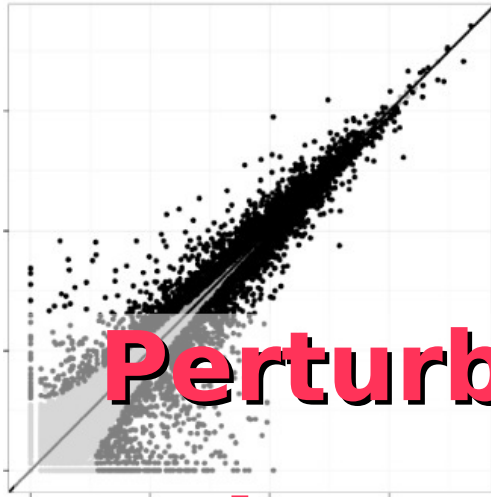




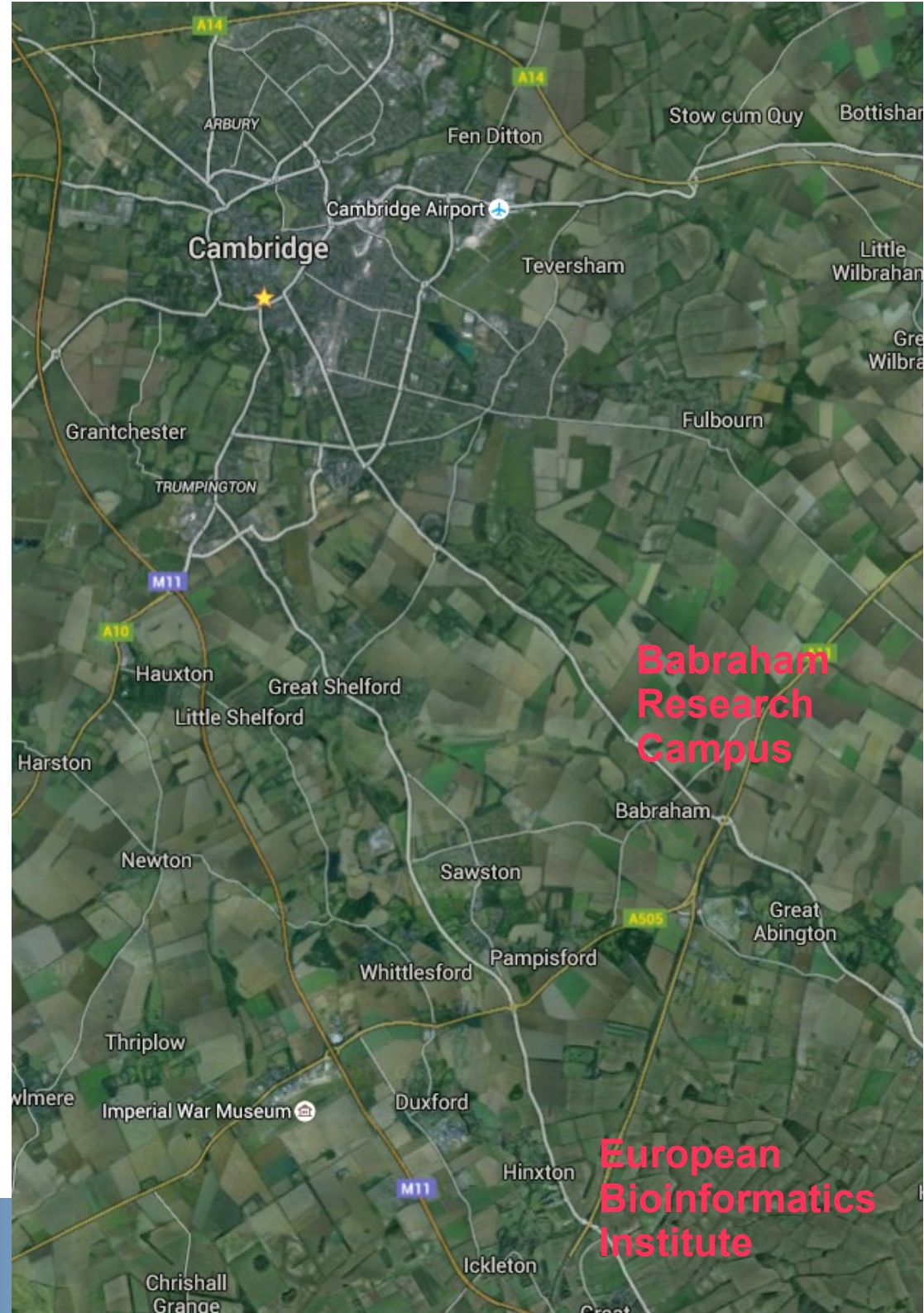
Perturbations of PIP3 signalling trigger a global remodelling of mRNA landscape and reveal a transcriptional feedback loop

Nicolas Le Novère
Babraham Institute,
n.lenovere@gmail.com



Programmes in:
Signalling
Immunology
Epigenetics
Nuclear Dynamics

Platforms including:
Bioinformatics
Imaging
FACS
Lipidomics
Mouse facilities
Sequencing



The Babraham Institute and the (phospho)lipids

- **Discovery of the liposome**

Bangham AD, Standish MM, Watkins JC (1965) Diffusion of univalent ions across the lamellae of swollen phospholipids. *J Mol Biol* 13, 238–252.

- **Discovery of IP3 signalling**

Berridge MJ and Irvine RF (1984) Inositol trisphosphate, a novel second messenger in cellular signal transduction. *Nature* 312, 315 – 321

- **Phosphorylation of PIP2 into PIP3 by PI3K**

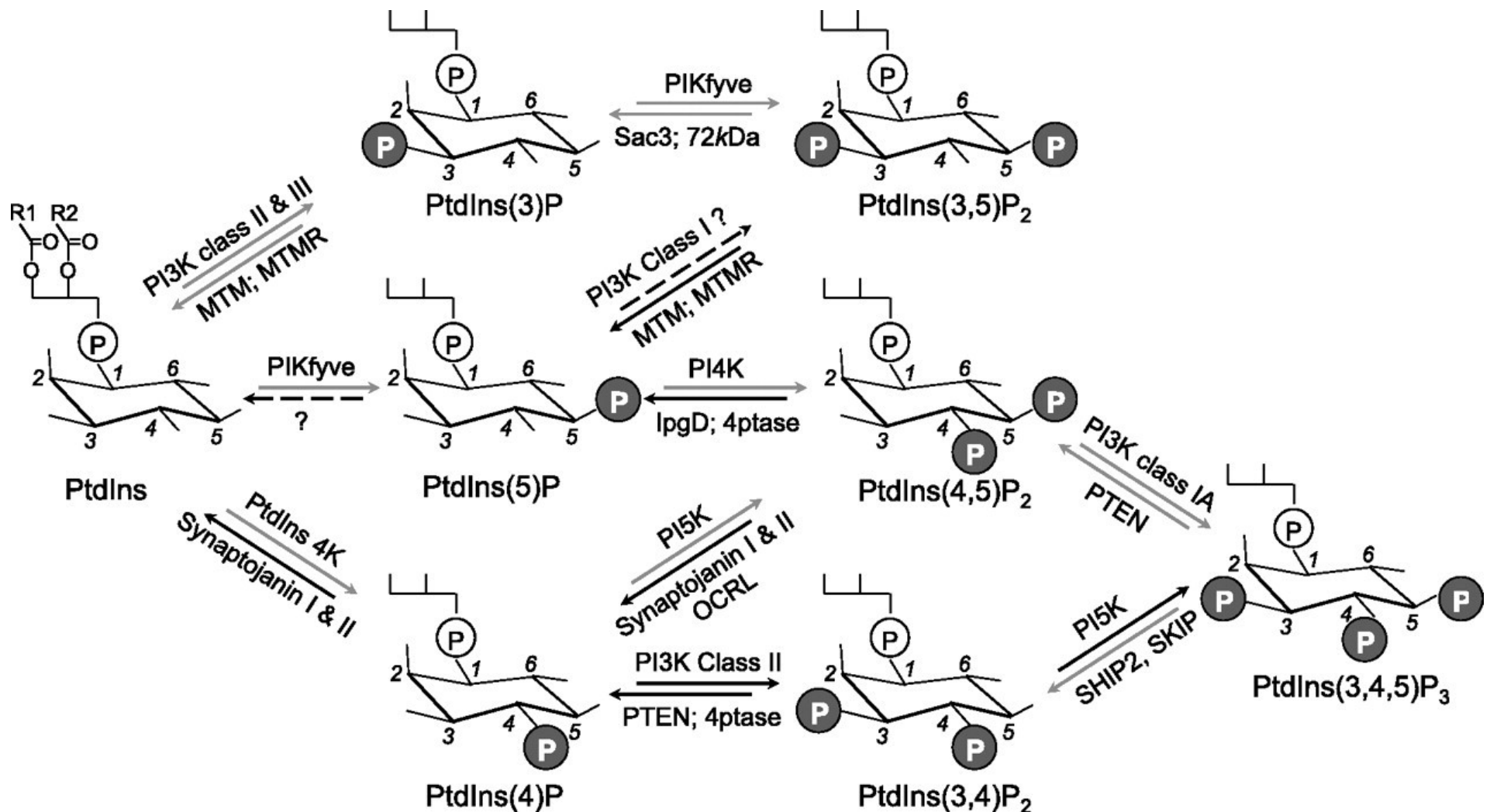
P.T. Hawkins, T.R. Jackson, L.R. Stephens (1992) Platelet-derived growth factor stimulates synthesis of PtdIns(3,4,5)P3 by activating a PtdIns(4,5)P2 3-OH kinase. *Nature* 358, 157-159

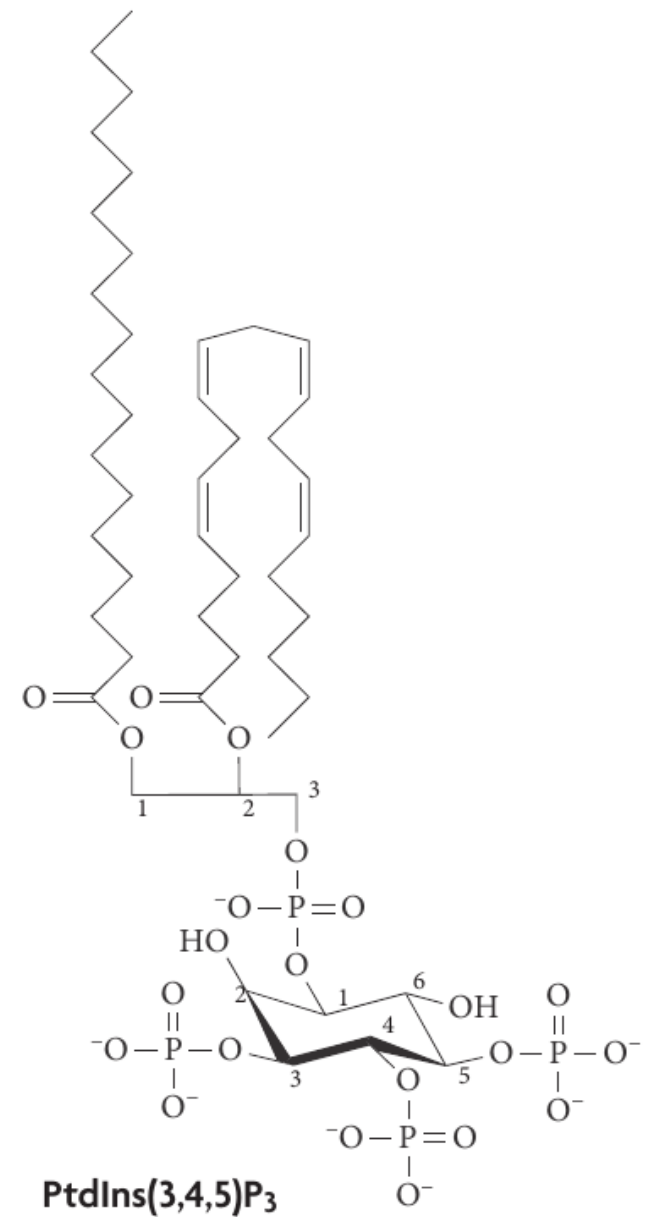
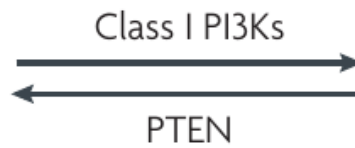
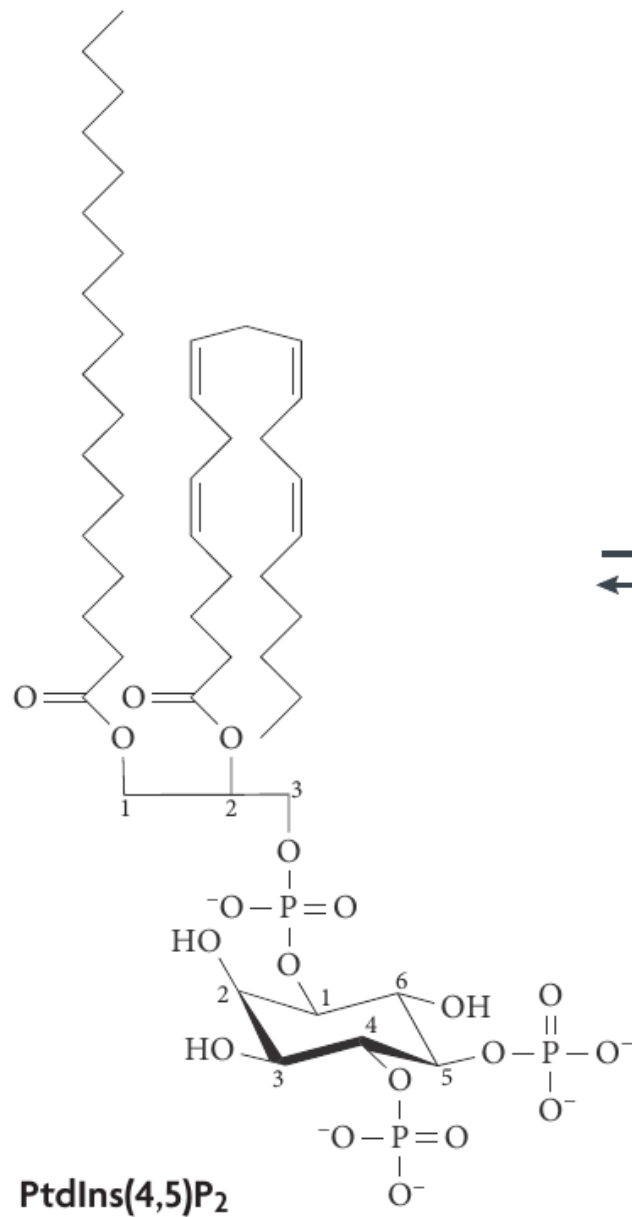
- **PIP3-dependent activation of PKB by PDK1**

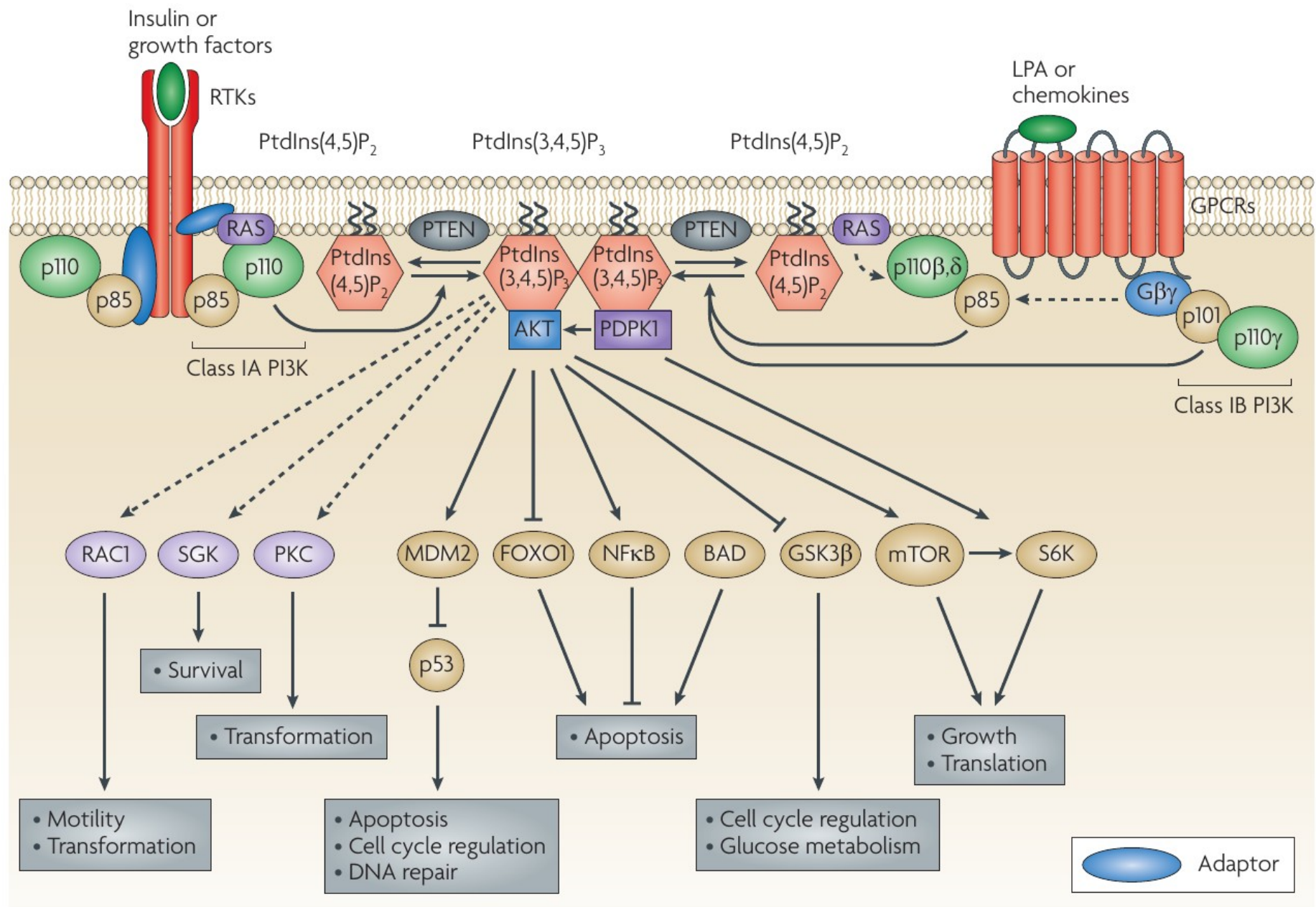
Stokoe D, Stephens LR, Copeland T, Gaffney PR, Reese CB, Painter GF, Holmes AB, McCormick F, Hawkins PT (1997) Dual role of phosphatidylinositol-3,4,5-trisphosphate in the activation of protein kinase B. *Science* 277, 567-570.

Stephens L.R., Anderson K., Stokoe D., Erdjument-Bromage H., Painter G.F., Holmes A.B., Gaffney P.R.J., Reese C.B., McCormick F., Tempst P., Coadwell J., Hawkins P.T. (1998) Protein Kinase B Kinases That Mediate Phosphatidylinositol 3,4,5-Trisphosphate-Dependent Activation of Protein Kinase B. *Science* 279, 710-714

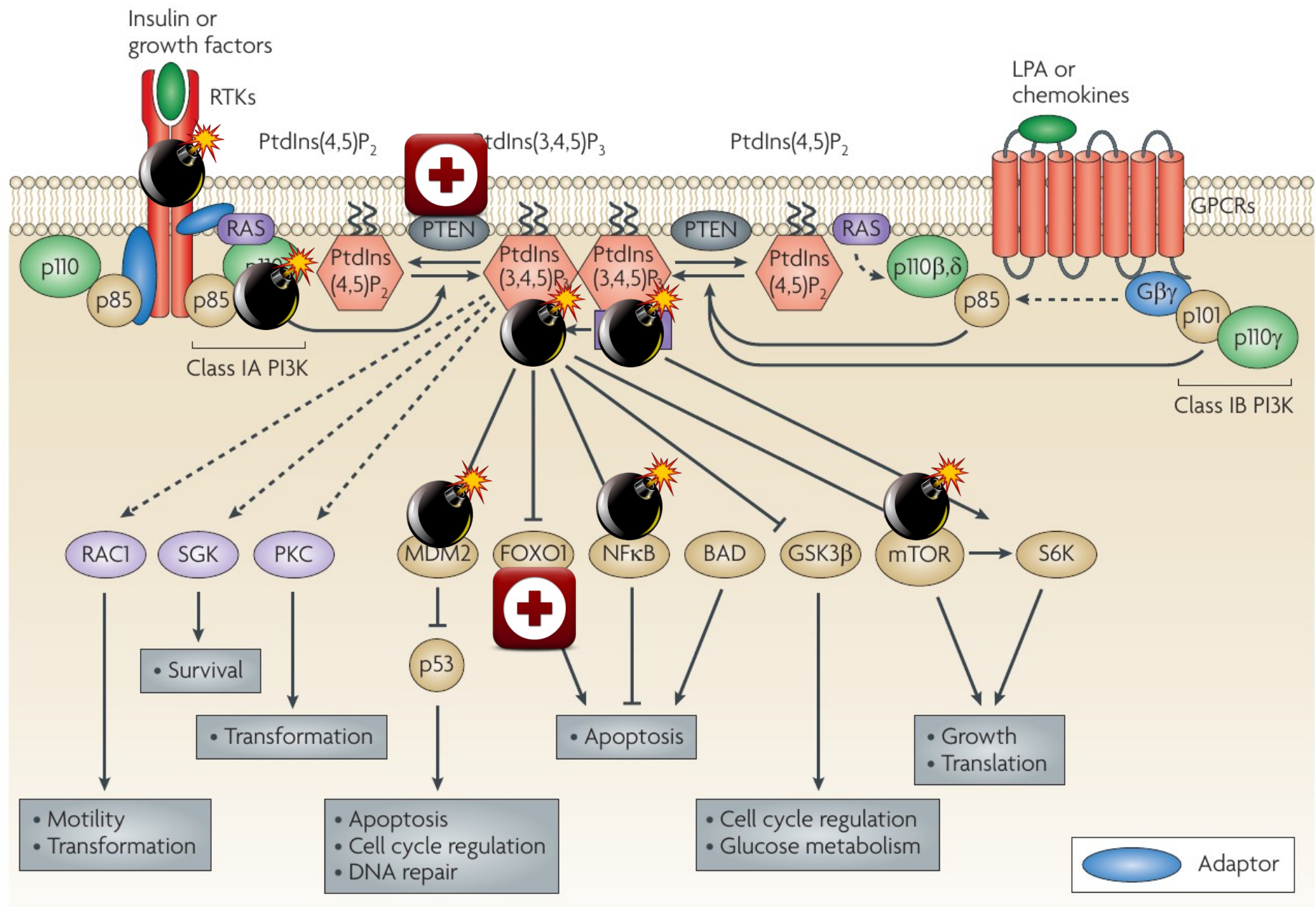
The phosphoinositides



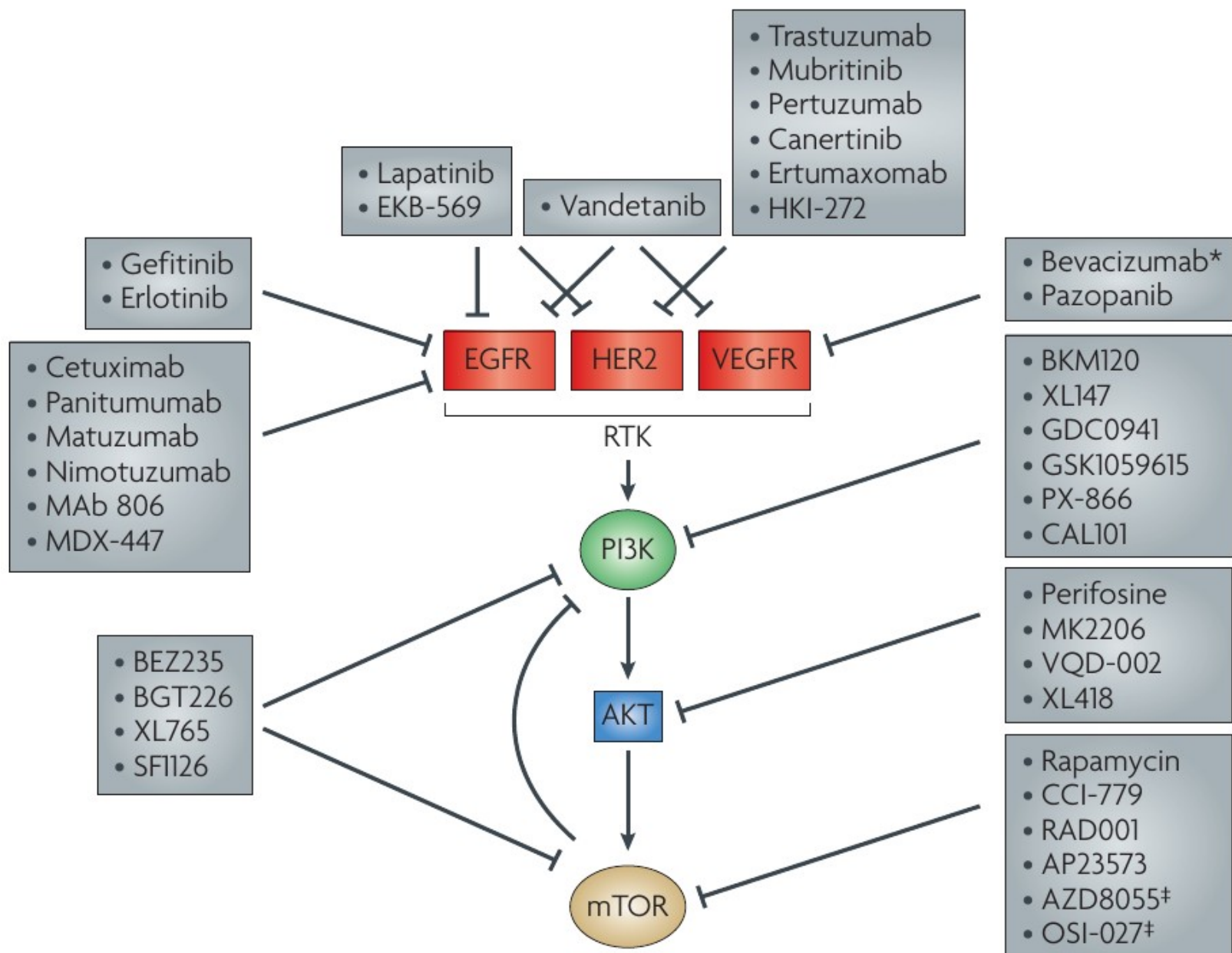




Liu *et al* (2009) *Nat Rev Drug Discov*

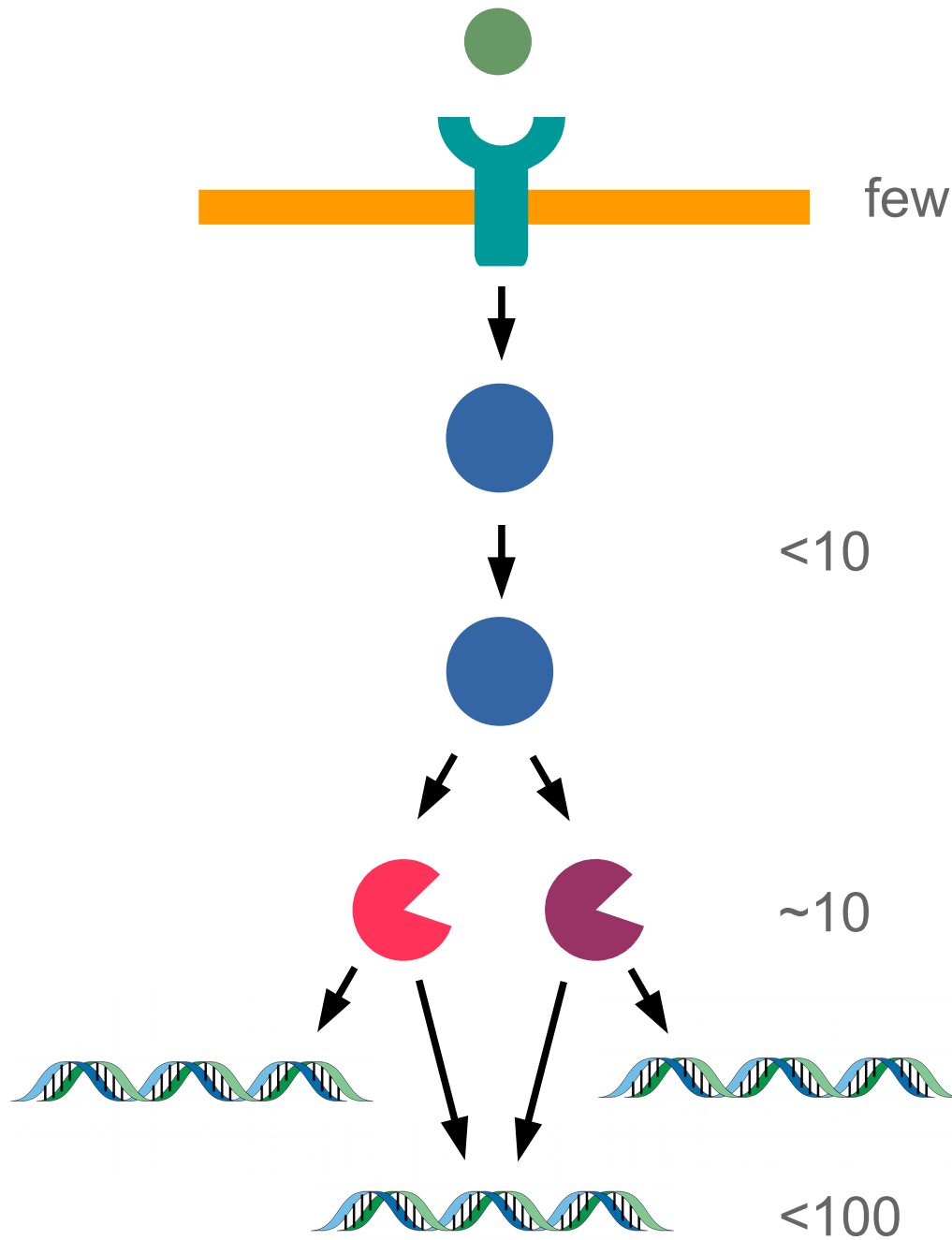


Liu *et al* (2009) *Nat Rev Drug Discov*

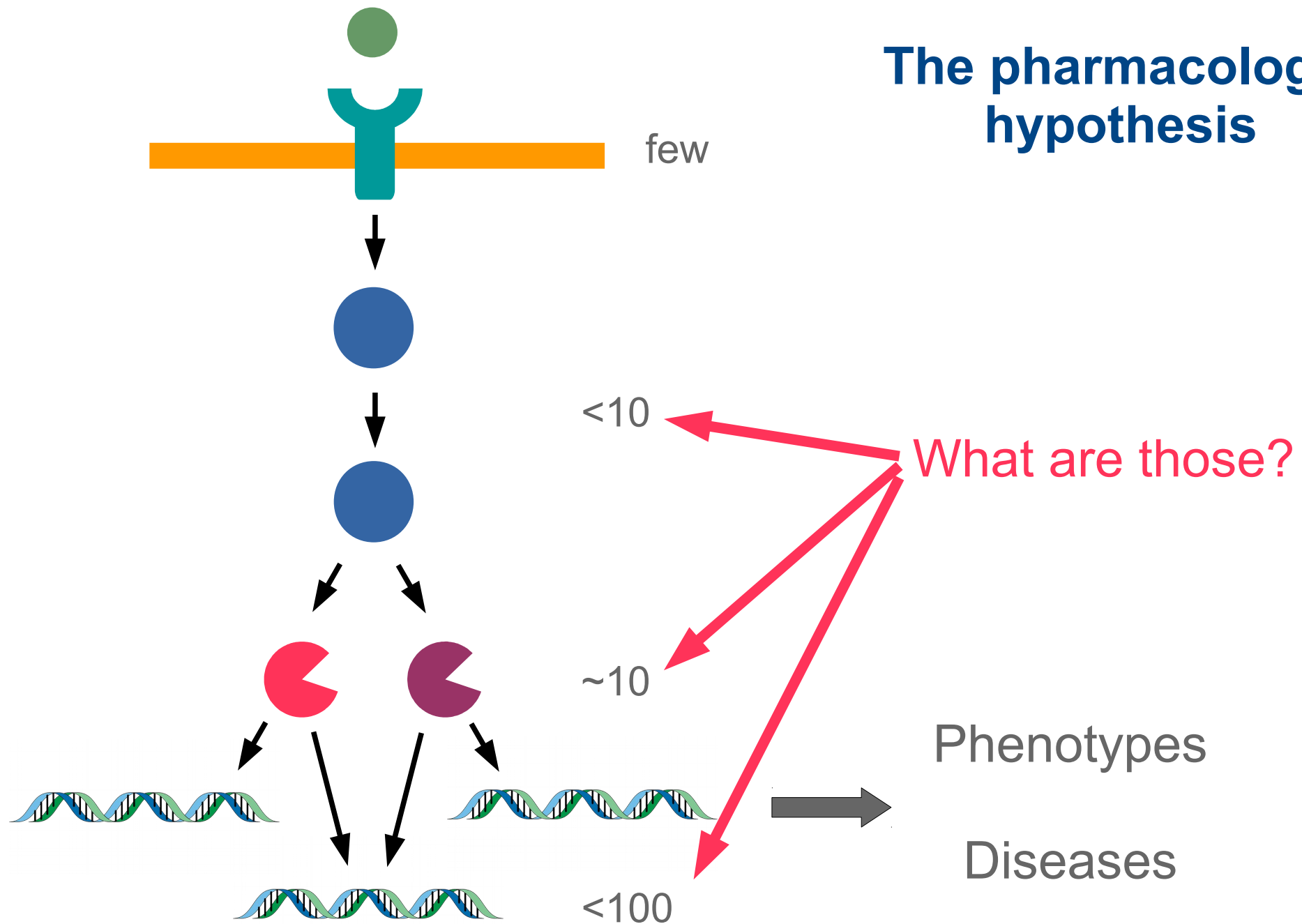


Liu *et al* (2009) *Nat Rev Drug Discov*

The pharmacology hypothesis

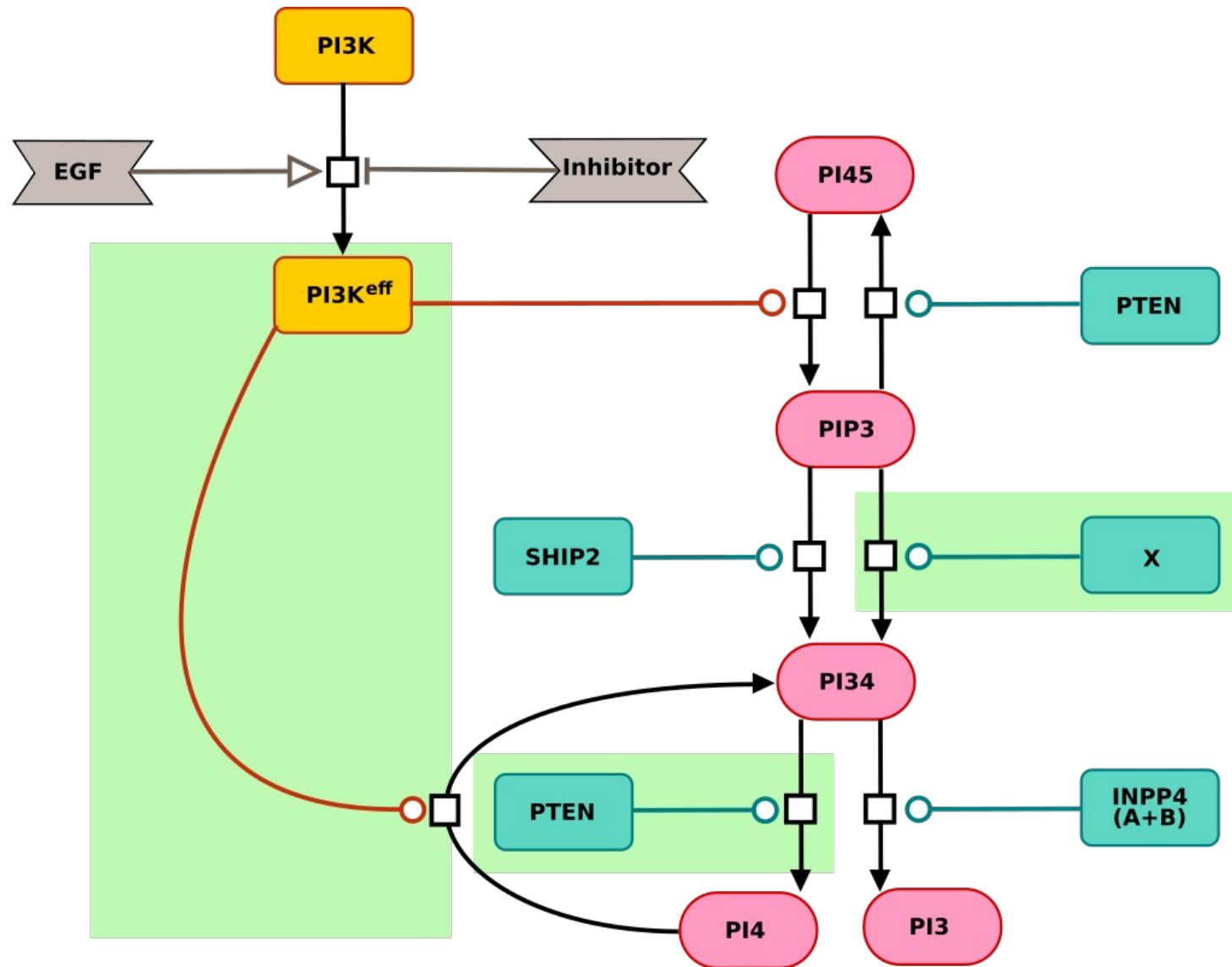


The pharmacology hypothesis



	experimental	computational
signalling	lipidomics (phosphoinositides mass-spectrometry)	chemical kinetic modelling
gene expression	transcriptomics (messenger RNA RNA-Seq)	clustering enrichment promoter analysis

Lipidomics and chemical kinetic modelling



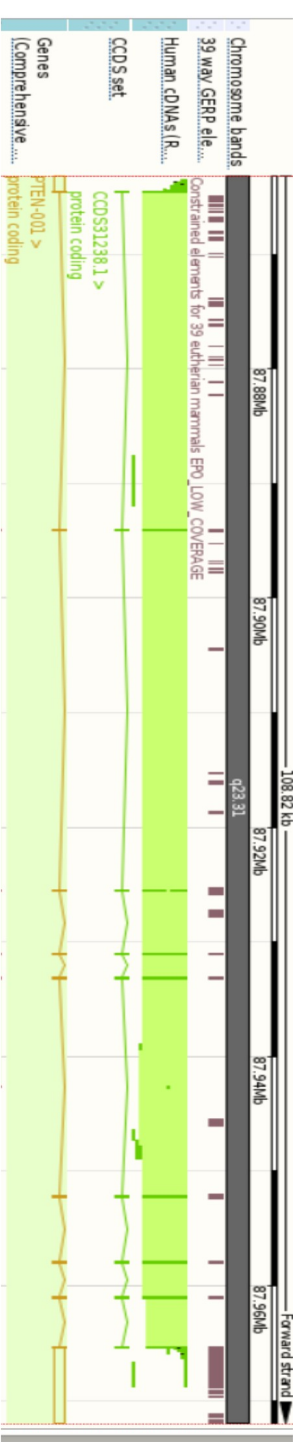


Vladimir Kiselev



Véronique Juvin

Kiselev, Juvin *et al* (2015) *Nucleic Acids Res*, gkv1015, ahead of print

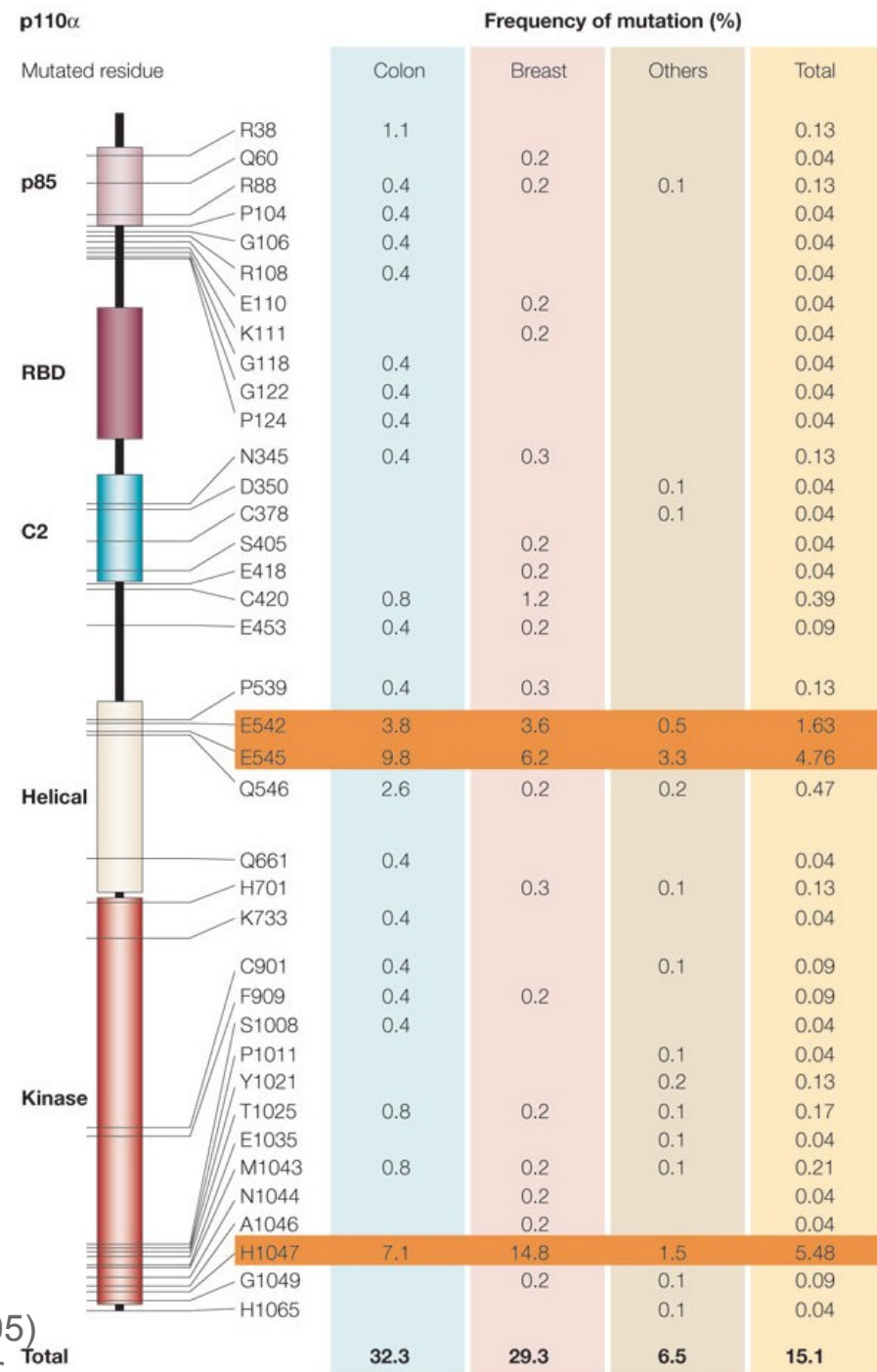


PTEN

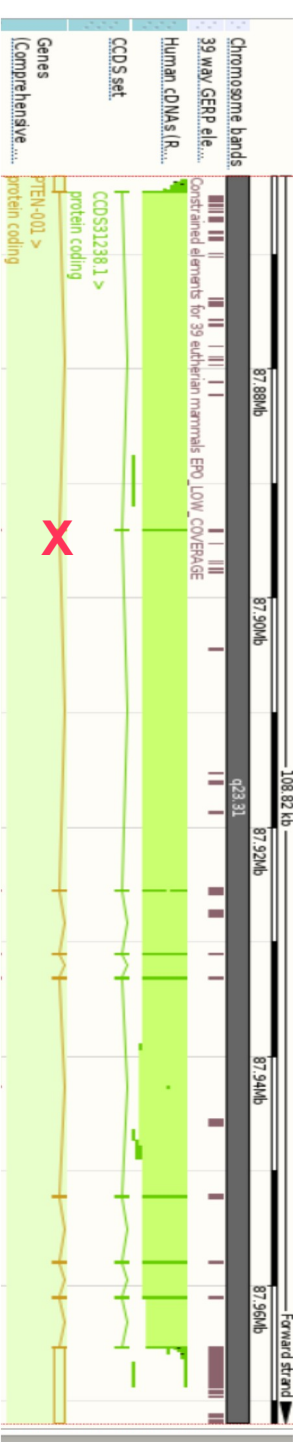
PI3K

Isogenic MCF10a cell lines

e!



Bader *et al* (2005)
Nat Rev Cancer



PTEN

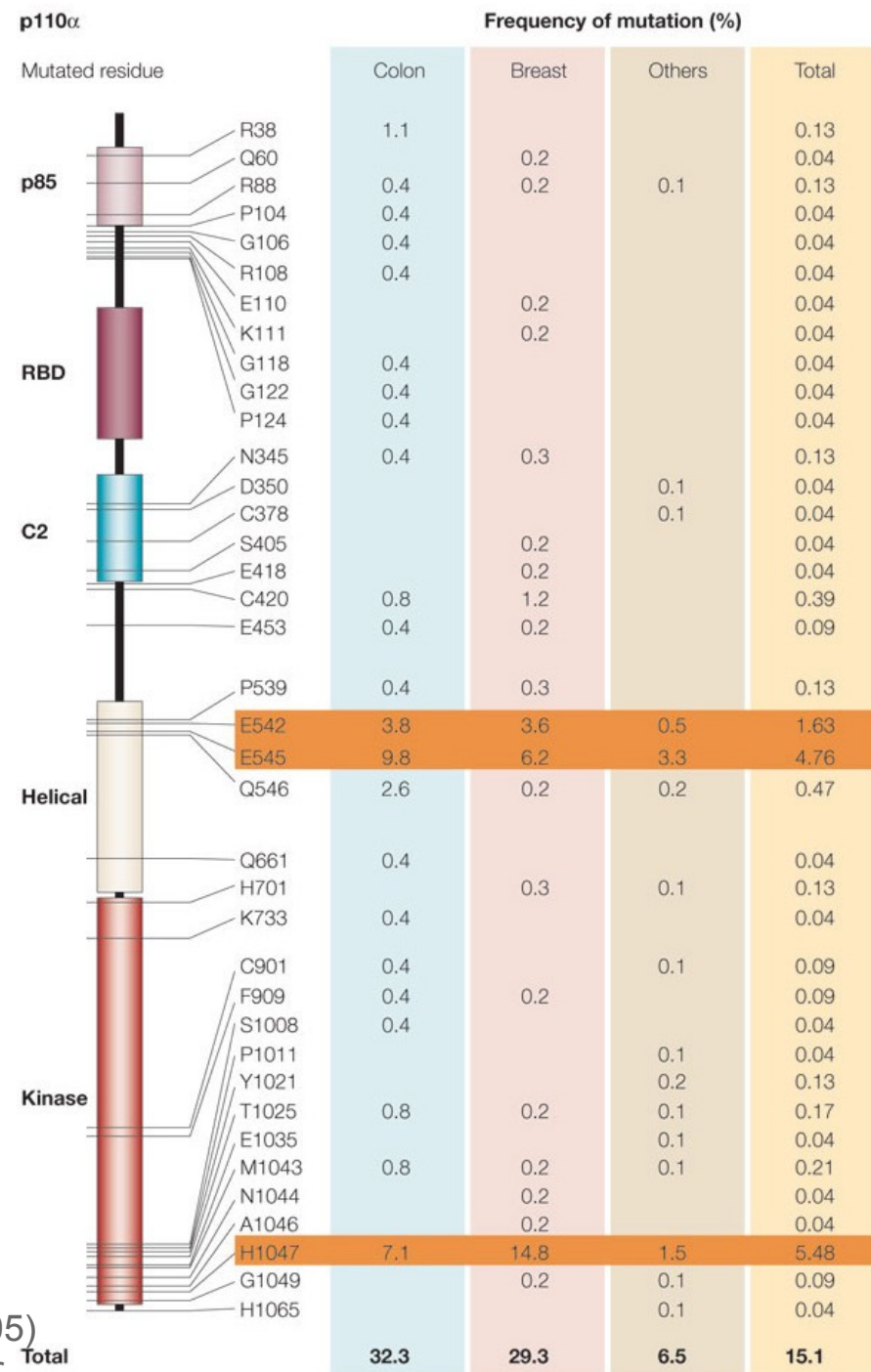
PI3K

**Isogenic MCF10a
cell lines**

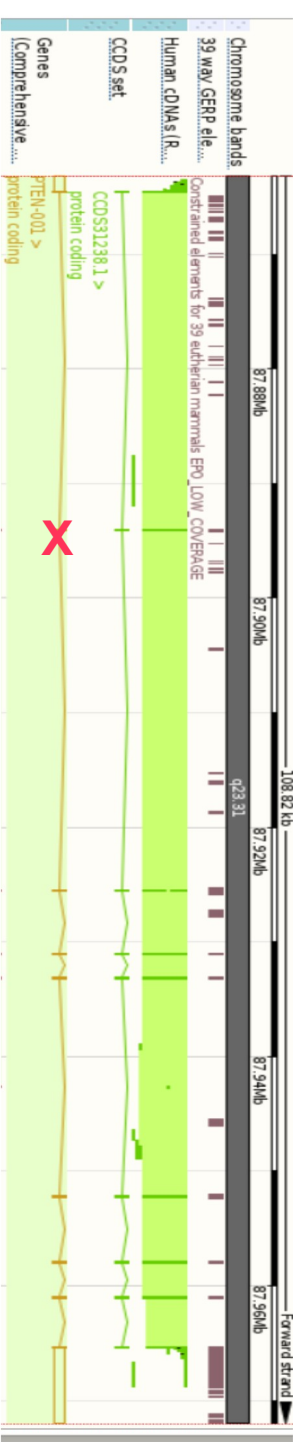
phosphatase
domain

inactivating
bi-allelic
mutation

e!



Bader *et al* (2005)
Nat Rev Cancer



PTEN

PI3K

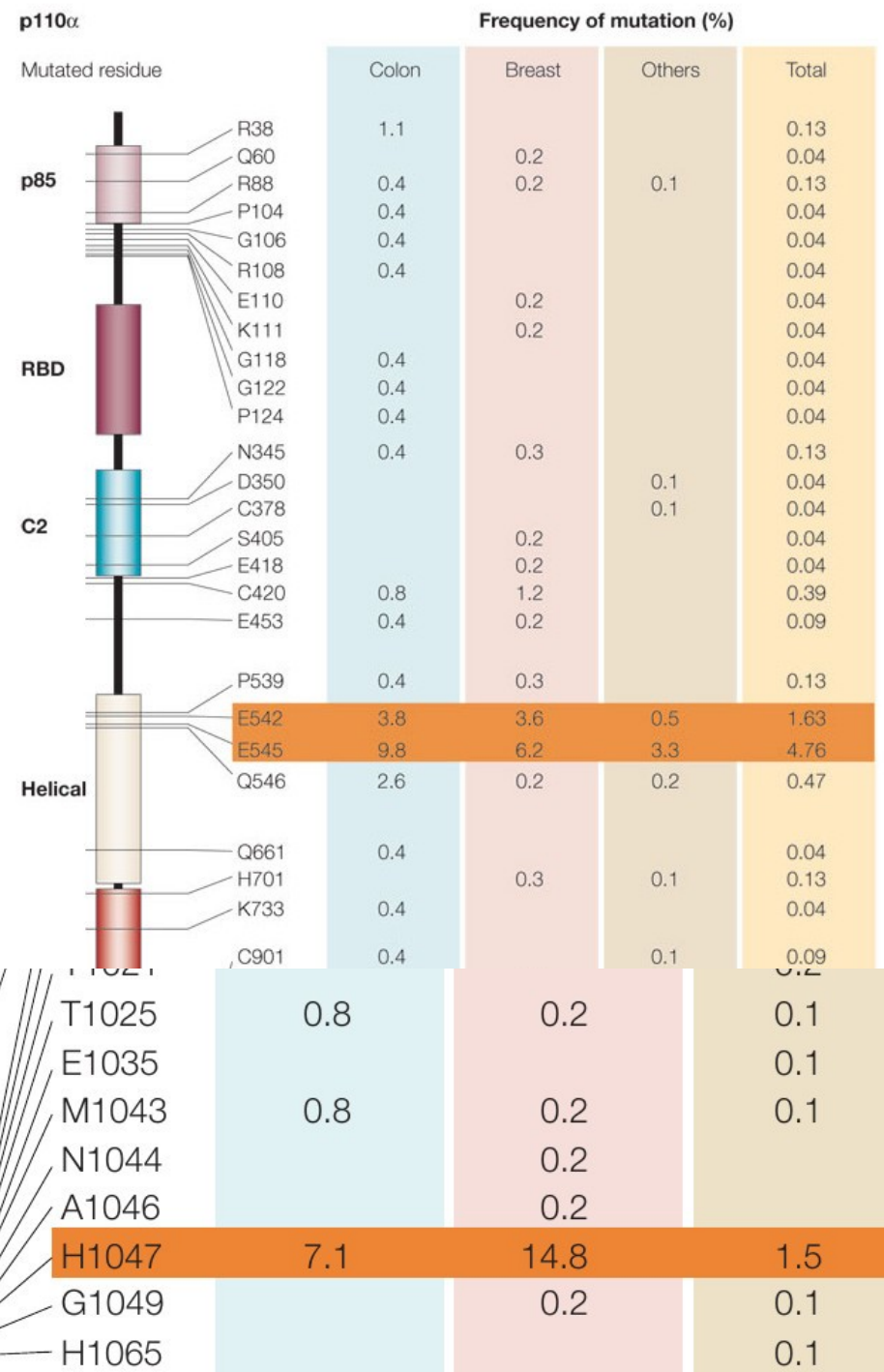
Isogenic MCF10a cell lines

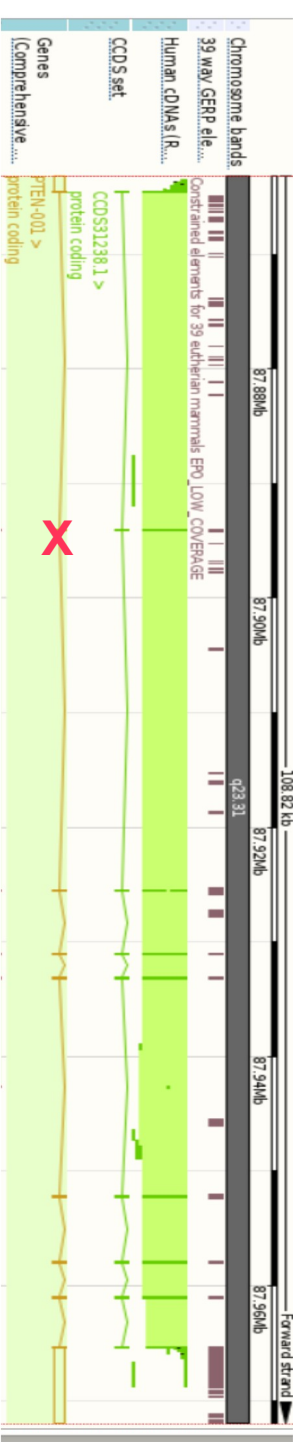
phosphatase domain

inactivating bi-allelic mutation

e!

activating mono-allelic mutation →





PTEN

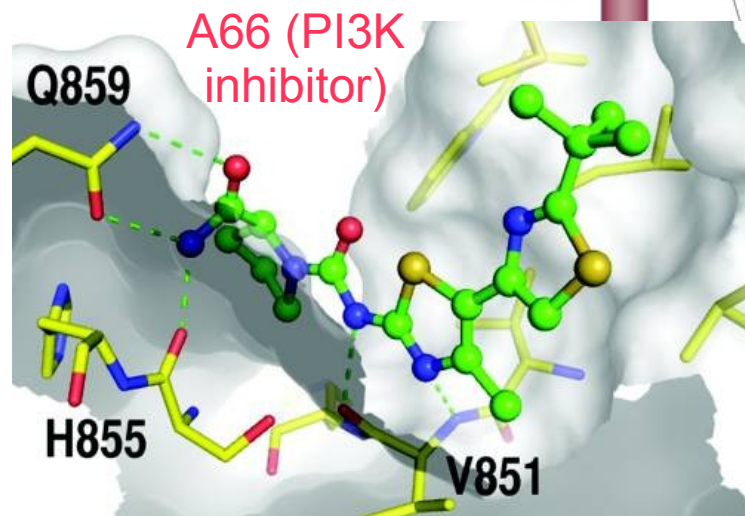
PI3K

**Isogenic MCF10a
cell lines**

phosphatase
domain

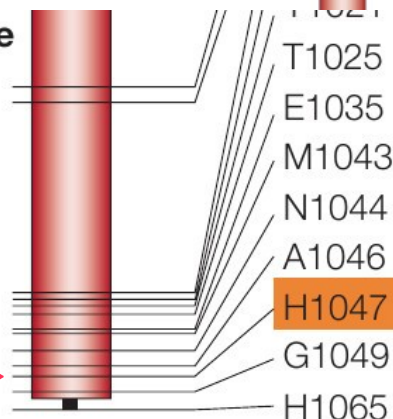
inactivating
bi-allelic
mutation

e!



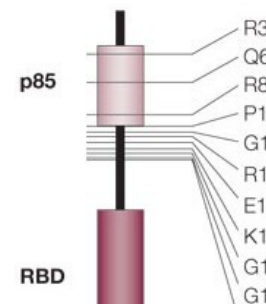
activating
mono-allelic
mutation →

Kinase



p110α

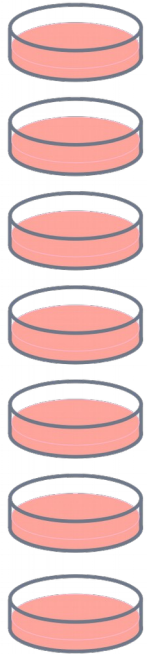
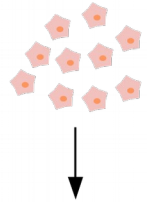
Mutated residue



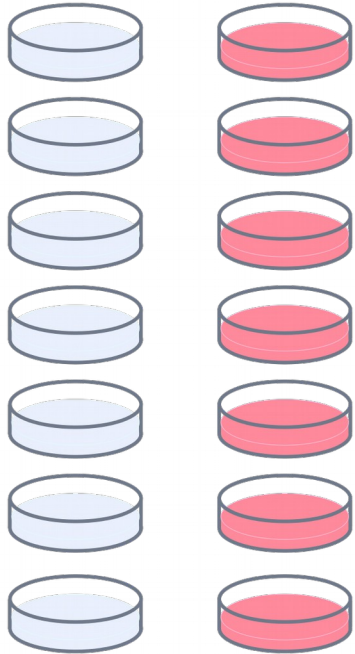
Frequency of mutation (%)

	Colon	Breast	Others	Total
R38	1.1			0.13
Q60		0.2		0.04
R88	0.4	0.2	0.1	0.13
P104	0.4			0.04
G106	0.4			0.04
R108	0.4			0.04
E110		0.2		0.04
K111		0.2		0.04
G118	0.4			0.04
G122	0.4			0.04
124	0.4			0.04
345	0.4	0.3		0.13
350			0.1	0.04
378			0.1	0.04
405		0.2		0.04
418		0.2		0.04
420	0.8	1.2		0.39
453	0.4	0.2		0.09
539	0.4	0.3		0.13
542	3.8	3.6	0.5	1.63
545	9.8	6.2	3.3	4.76
546	2.6	0.2	0.2	0.47
661	0.4			0.04
701		0.3	0.1	0.13
K733	0.4			0.04
C901	0.4		0.1	0.09
T1025	0.8	0.2		0.1
E1035				0.1
M1043	0.8	0.2		0.1
N1044		0.2		
A1046		0.2		
H1047	7.1	14.8		1.5
G1049		0.2		0.1
H1065				0.1

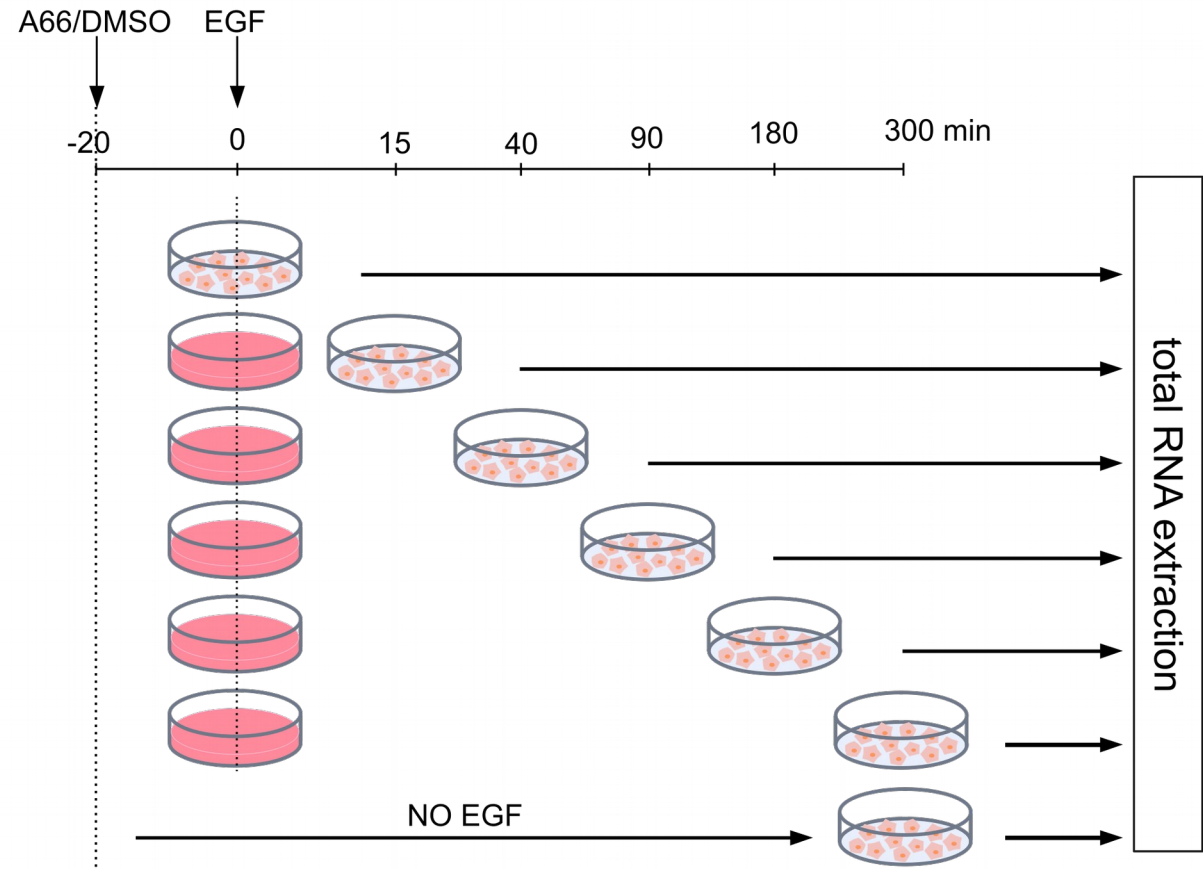
Day 1. Seed the cells



Day 2. Wash and Starve



Day 3. Drugs and RNA extraction



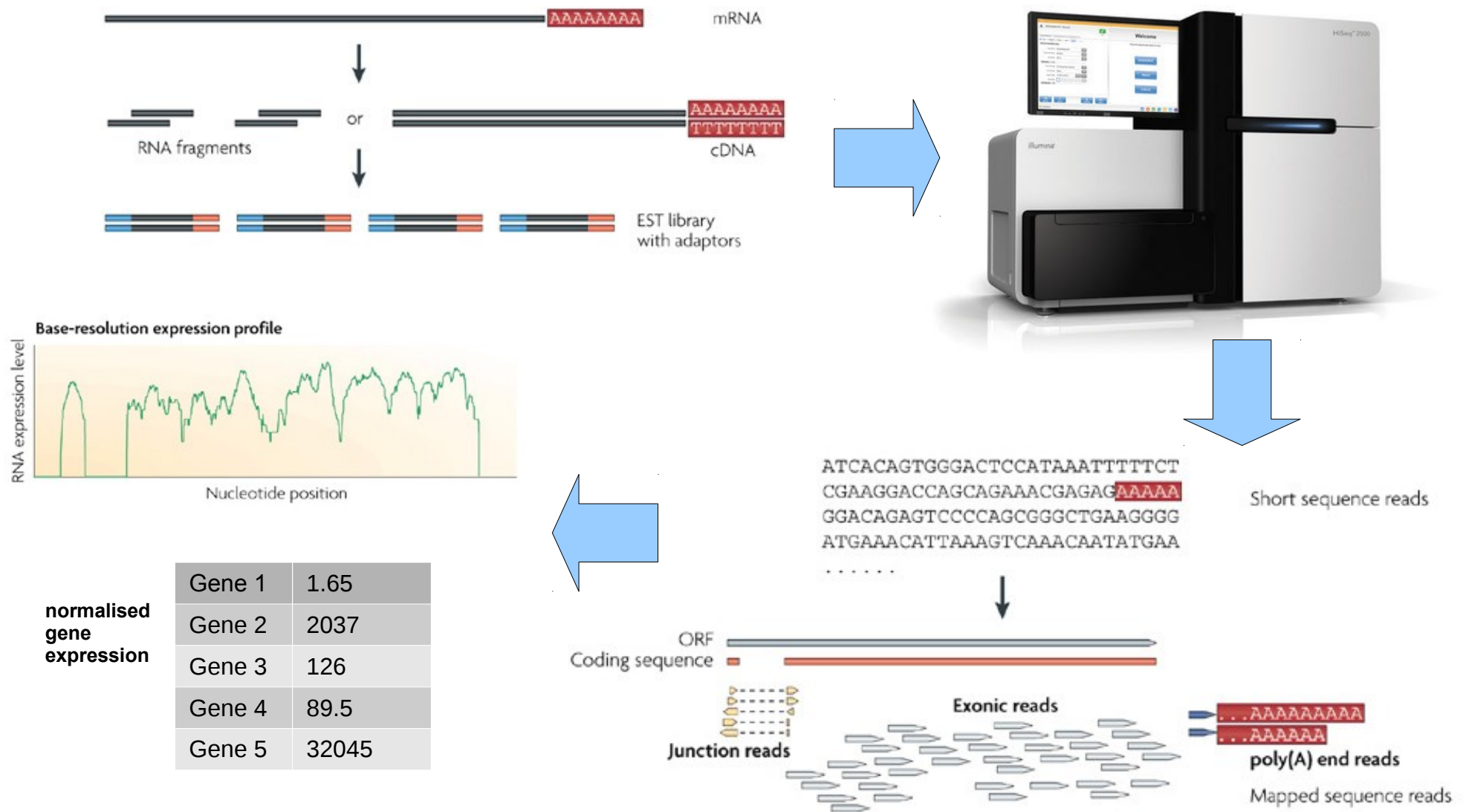
Growth medium

PBS

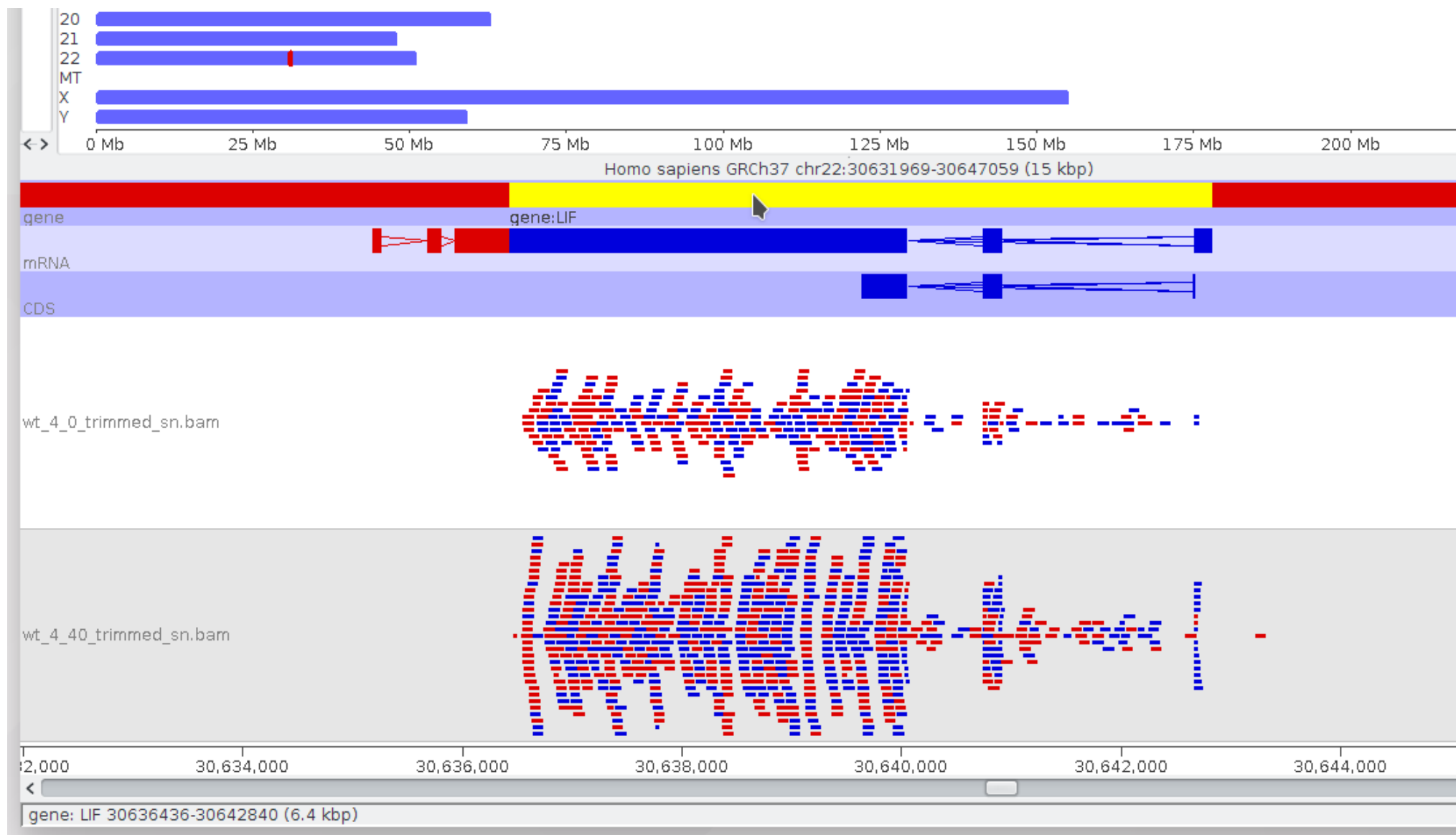
Assay medium- no EGF
(except for stimulation)

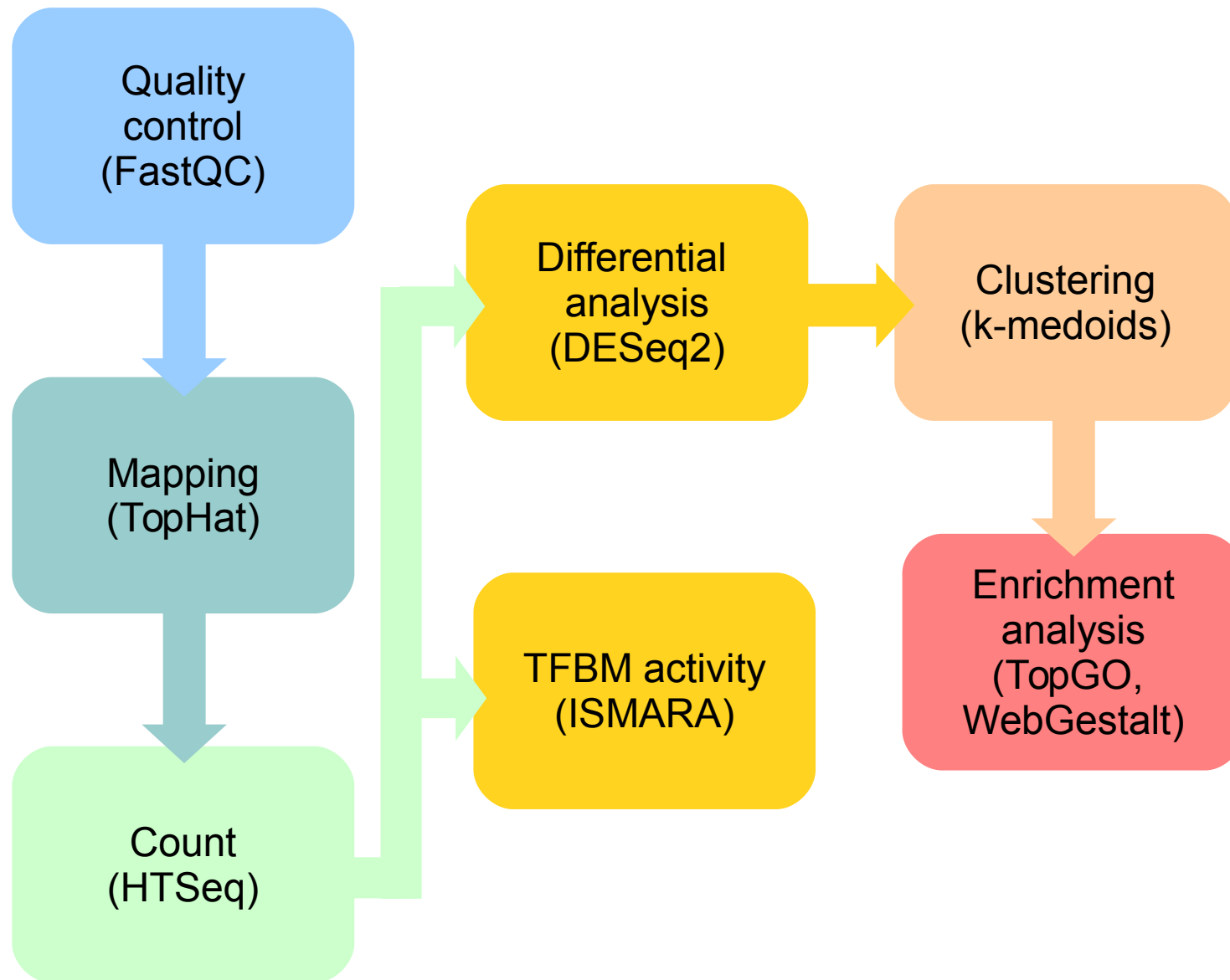
Lysis Buffer

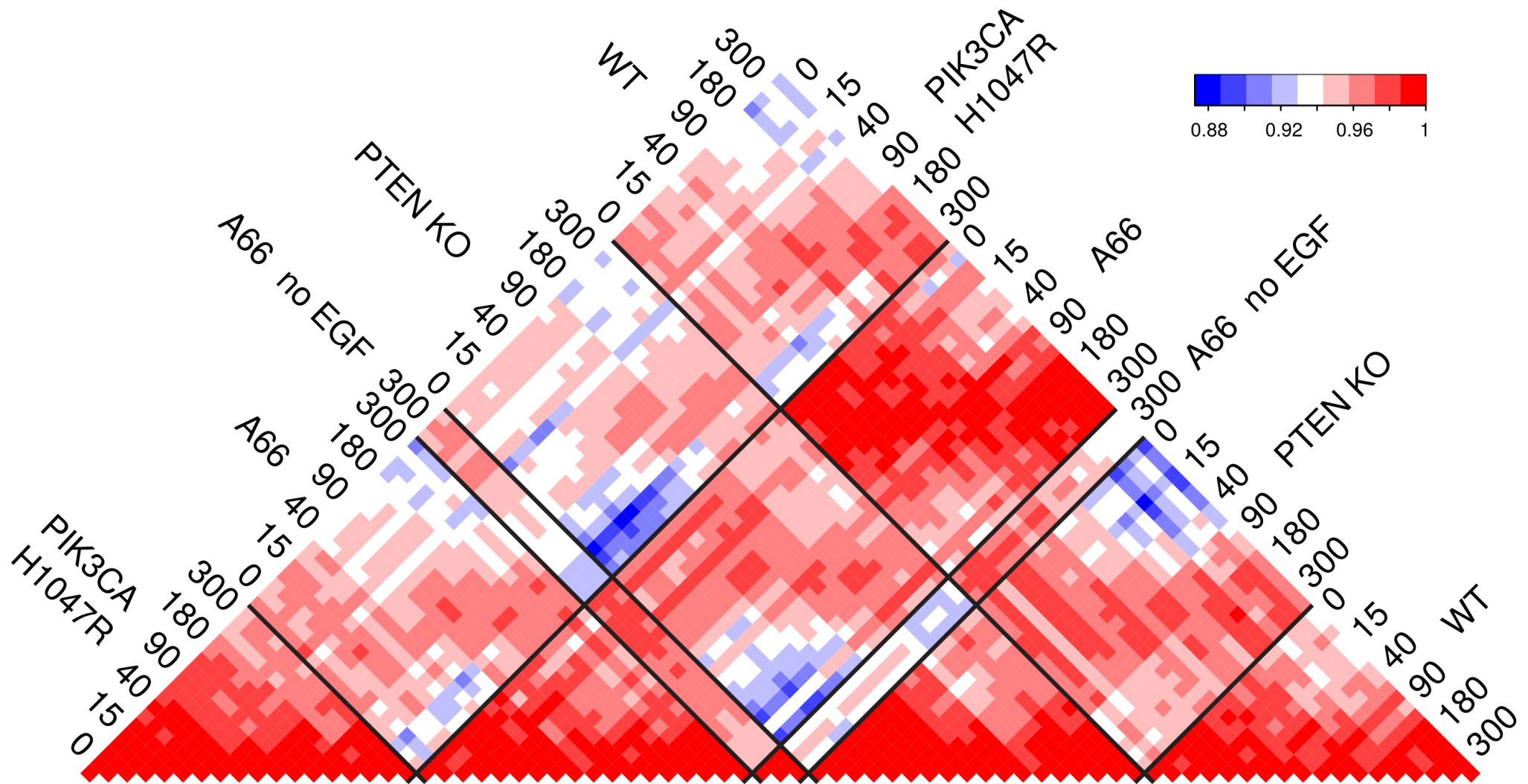
Measuring gene expression with RNA-Seq



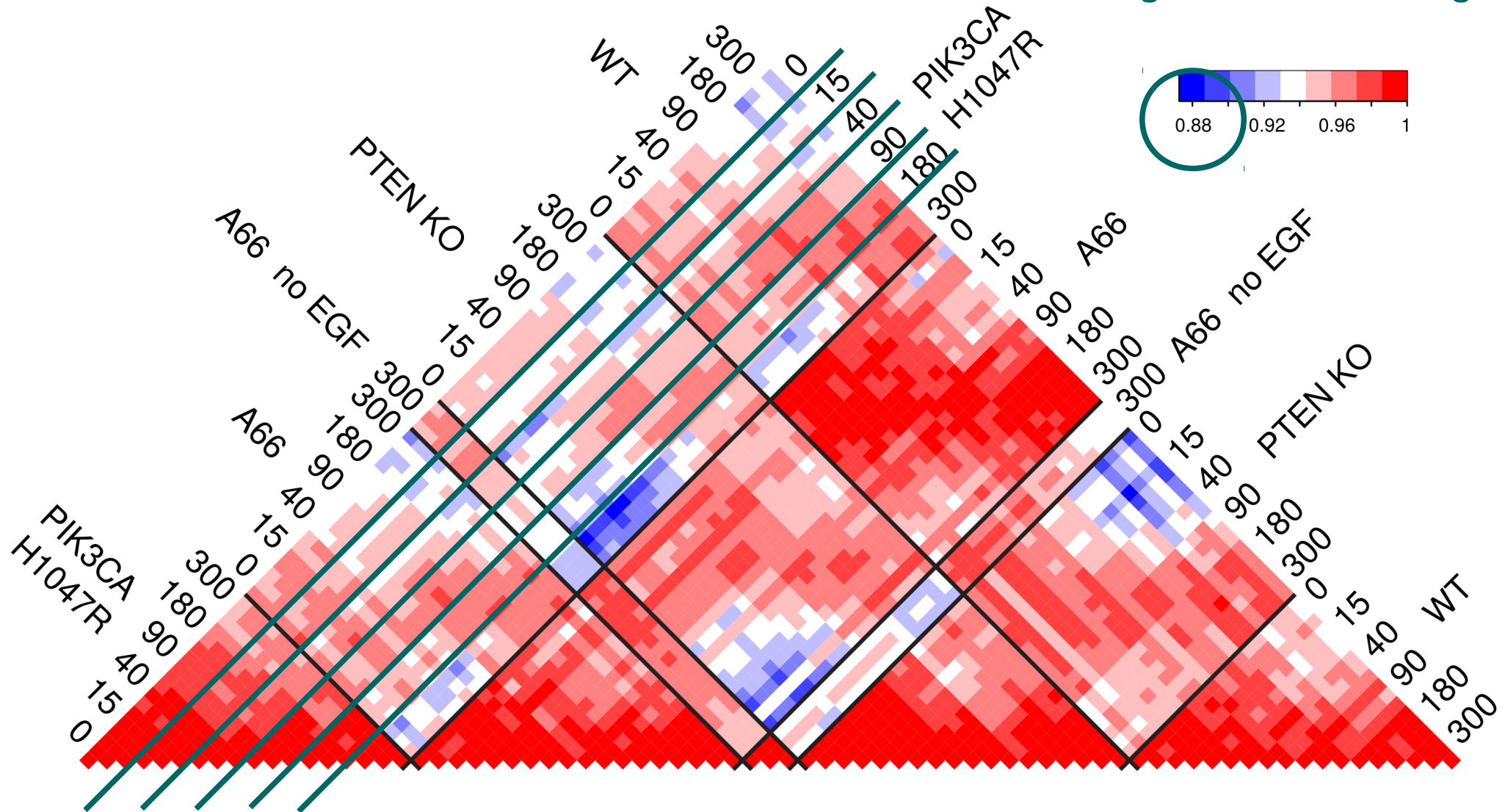
Wang *et al* (2009) *Nat Rev Genet*





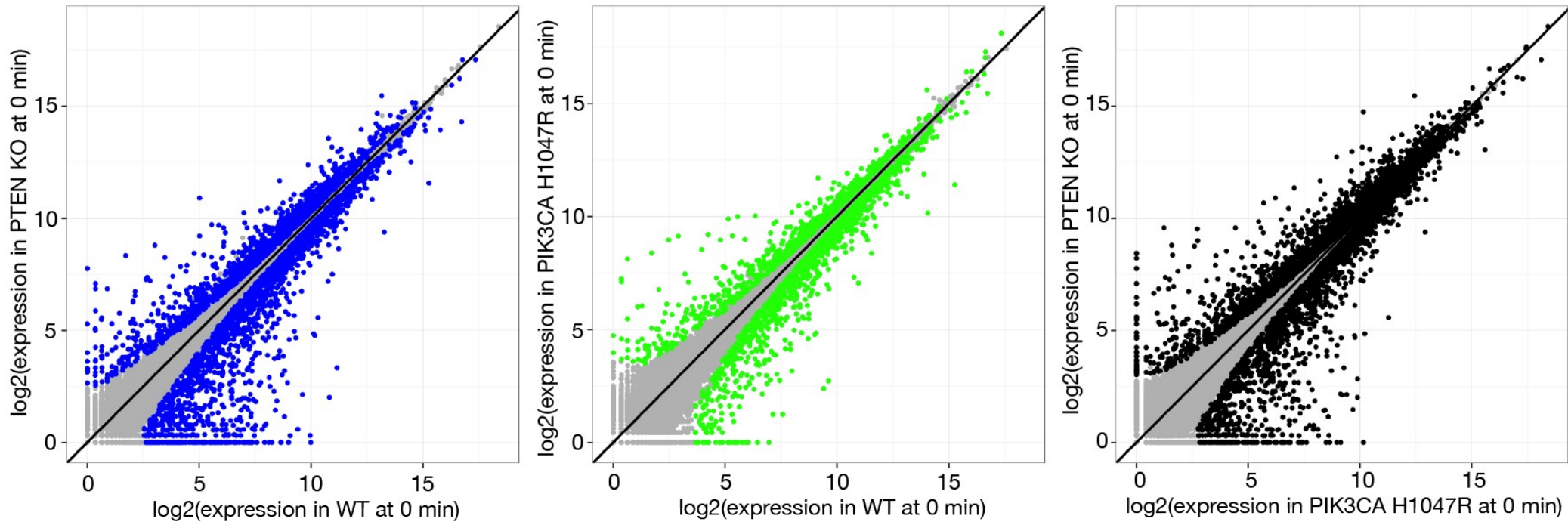


Most genes do not change



replicates are OK

But quite a few are affected nevertheless



4725 genes affected by A66

1543 genes affected by H1047R

2244 genes affected by PTEN-/-

The butterfly effect in cancer: A single base mutation can remodel the cell

Jonathan R. Hart^a, Yaoyang Zhang^b, Lujian Liao^b, Lynn Ueno^a, Lisa Du^a, Marloes Jonkers^a, John R. Yates III^b, and Peter K. Vogt^{a,1}

Departments of ^aMolecular and Experimental Medicine and ^bChemical Physiology, The Scripps Research Institute, La Jolla, CA 92037

Contributed by Peter K. Vogt, December 15, 2014 (sent for review August 11, 2014)

We have compared the proteome, transcriptome, and metabolome of two cell lines: the human breast epithelial line MCF-10A and its mutant descendant MCF-10A-H1047R. These cell lines are derived from the same parental stock and differ by a single amino acid substitution (H1047R) caused by a single nucleotide change in one allele of the PIK3CA gene, which encodes the catalytic subunit p110 α of PI3K (phosphatidylinositol 3-kinase). They are considered isogenic. The H1047R mutation of PIK3CA is one of the most frequently encountered somatic cancer-specific mutations. In MCF-10A, this mutation induces an extensive cellular reorganization that far exceeds the known signaling activities of PI3K. The changes are highly diverse, with examples in structural protein levels, the DNA repair machinery, and sterol synthesis. Gene set enrichment analysis reveals a highly significant concordance of the genes differentially expressed in MCF-10A-H1047R cells and the established protein and RNA signatures of basal breast cancer. No such concordance was found with the specific gene signatures of other histological types of breast cancer. Our data document the power of a single base mutation, inducing an extensive remodeling of the cell toward the phenotype of a specific cancer.

RNAseq | SILAC | knock-in | molecular signature | basal breast cancer

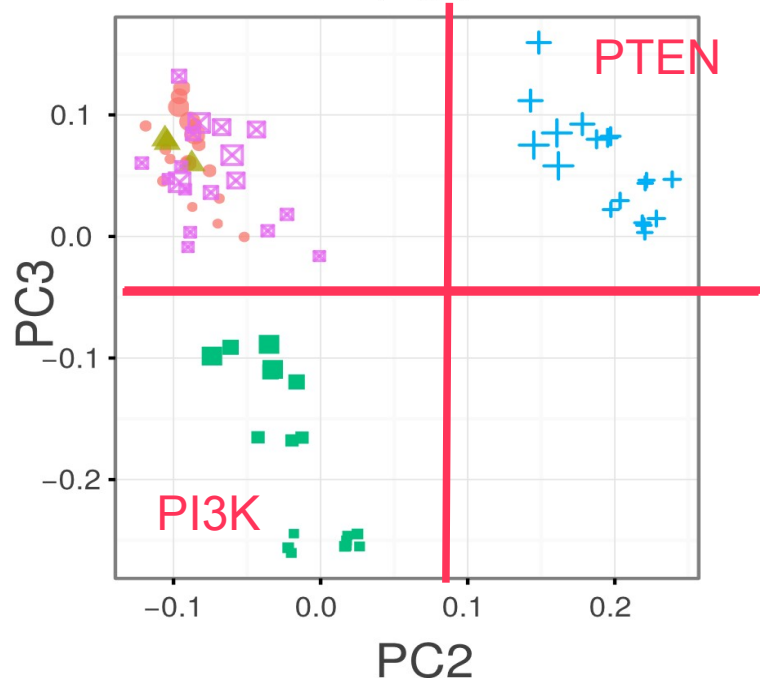
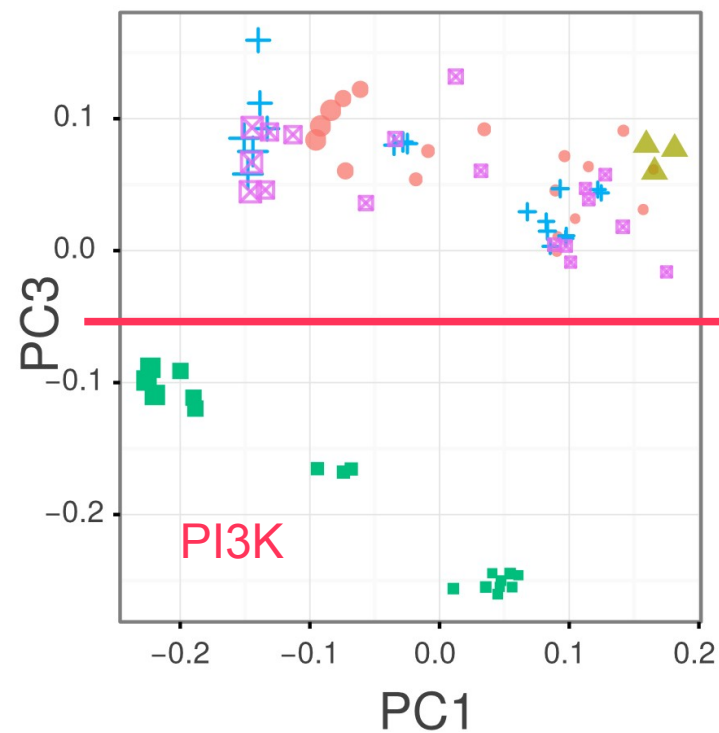
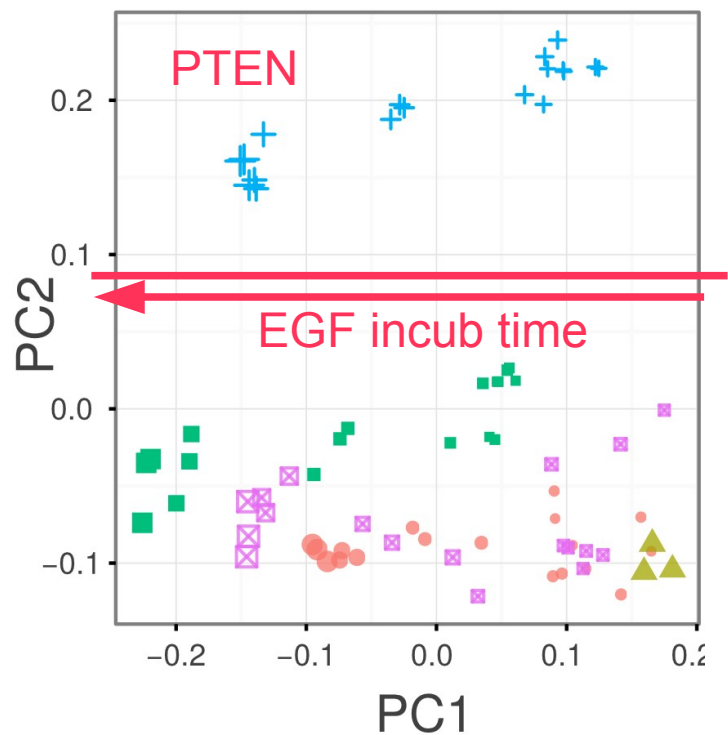
MCF-10A and MCF-10A-H1047R can grow in chemically defined, serum-free medium, facilitating the amino acid substitutions required by SILAC and avoiding the variability introduced by the use of serum in the culture medium (7, 9).

The changes induced in protein and RNA expression by the H1047R mutation document a comprehensive reorganization of the cell, including a shift of the expression patterns toward the signature of basal breast cancer.

Results

Genetic Comparison of the MCF-10A and MCF-10A-H1047R Cell Lines.

MCF-10A and MCF-10A-H1047R are considered isogenic, except for the knock-in mutation of H1047R in one allele of PIK3CA. However, during the creation of the H1047R knock-in or in the course of the subsequent culture, other mutations in cancer-relevant genes could have been introduced or selected for. To investigate this possibility, both cell lines were studied by whole-exome sequencing. The procedures used for exome sequencing are described in *SI Materials and Methods*. This sequence information was used to determine variant SNPs (single-nucleotide polymorphisms) and insertions and deletions, as well as copy number variations. Variants that are significantly different between the two cell lines are shown in *Table S1*. Other



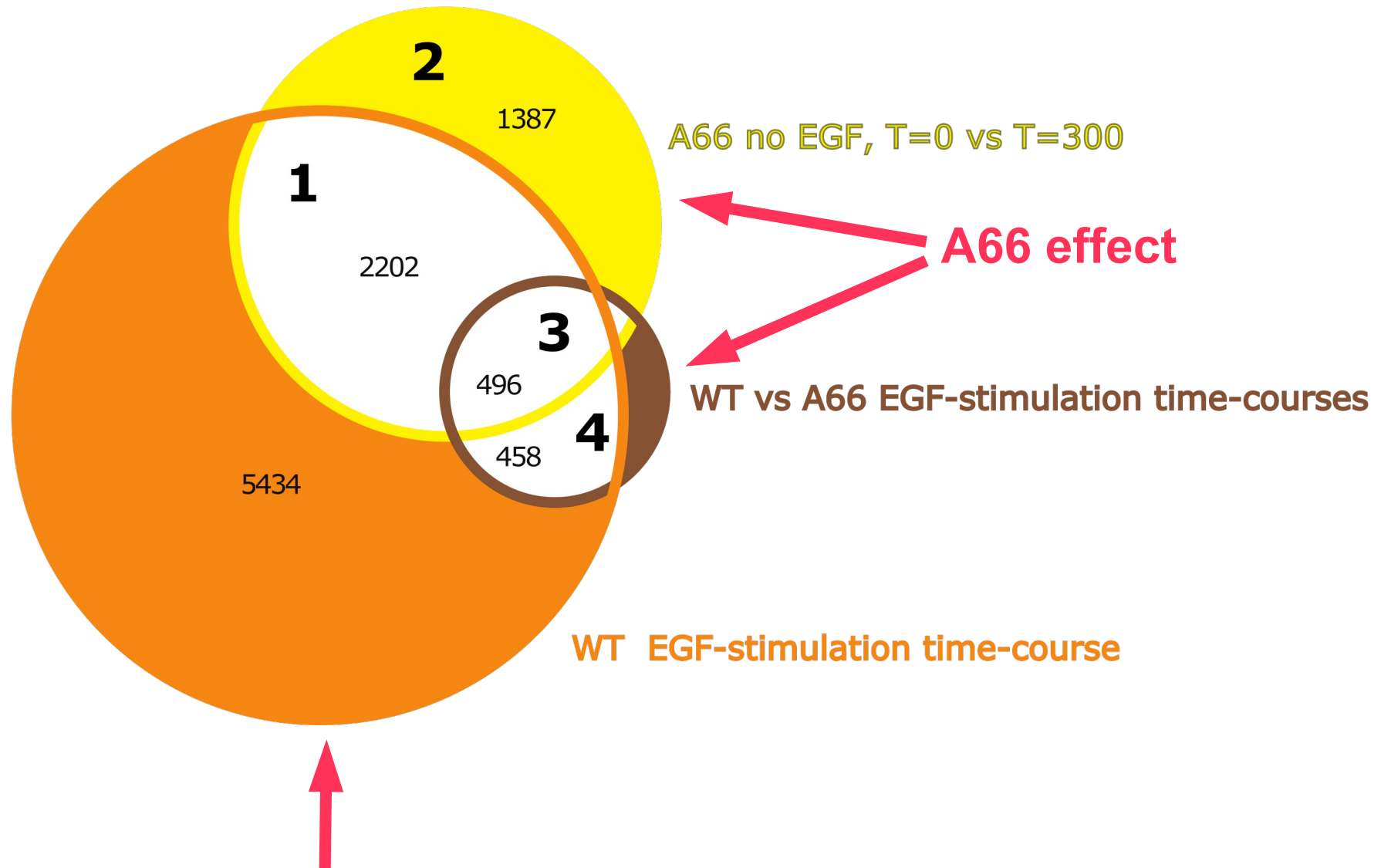
Time, min

- 0
- 100
- 200
- 300

Condition

- ⊠ WT
- A66
- ▲ A66 no EGF
- PIK3CA H1047R
- + PTEN KO

Effect of acute PI3K inhibition

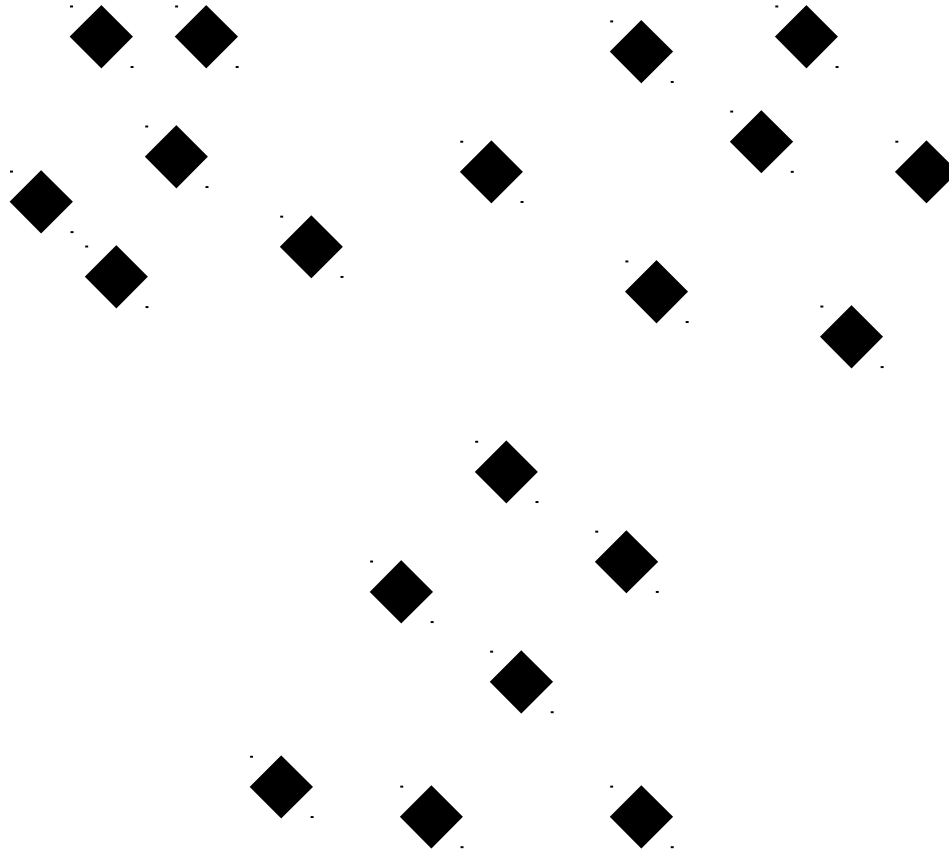


Most EGF effects are not PI3K-dependent (MAPK etc.)

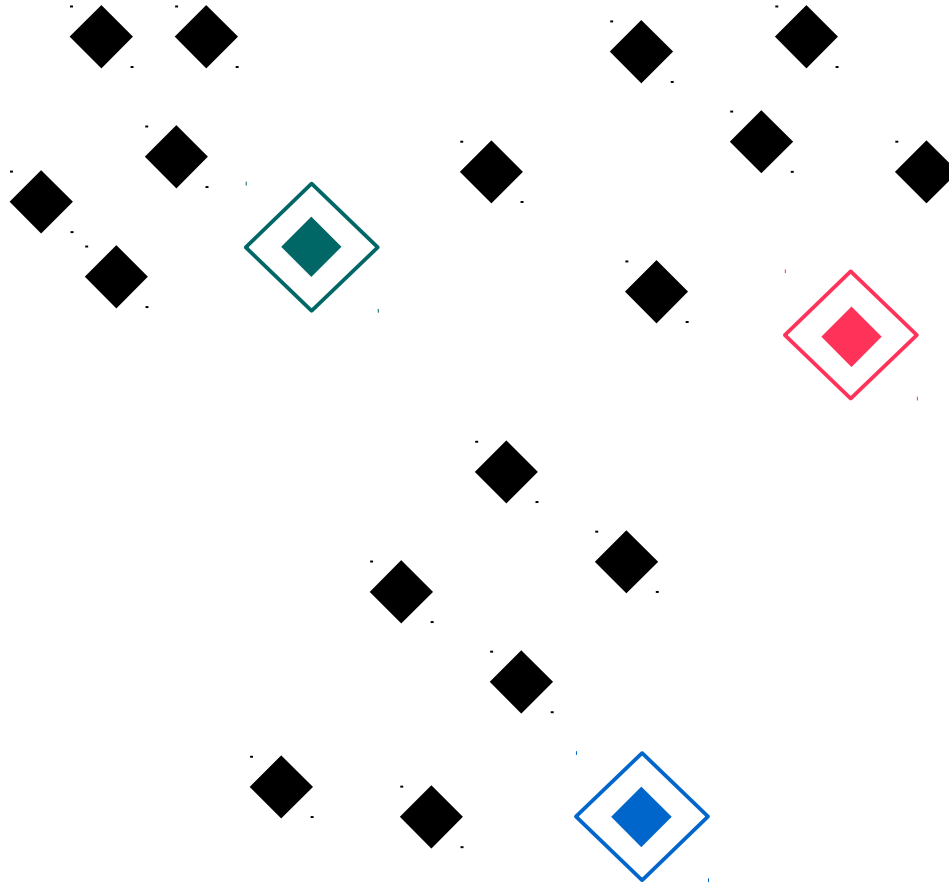
Clustering by K-medoid method

- Try 2 to n medoids, n depending on the number of samples
- Compute the stability of clusters with 100 data bootstrappings

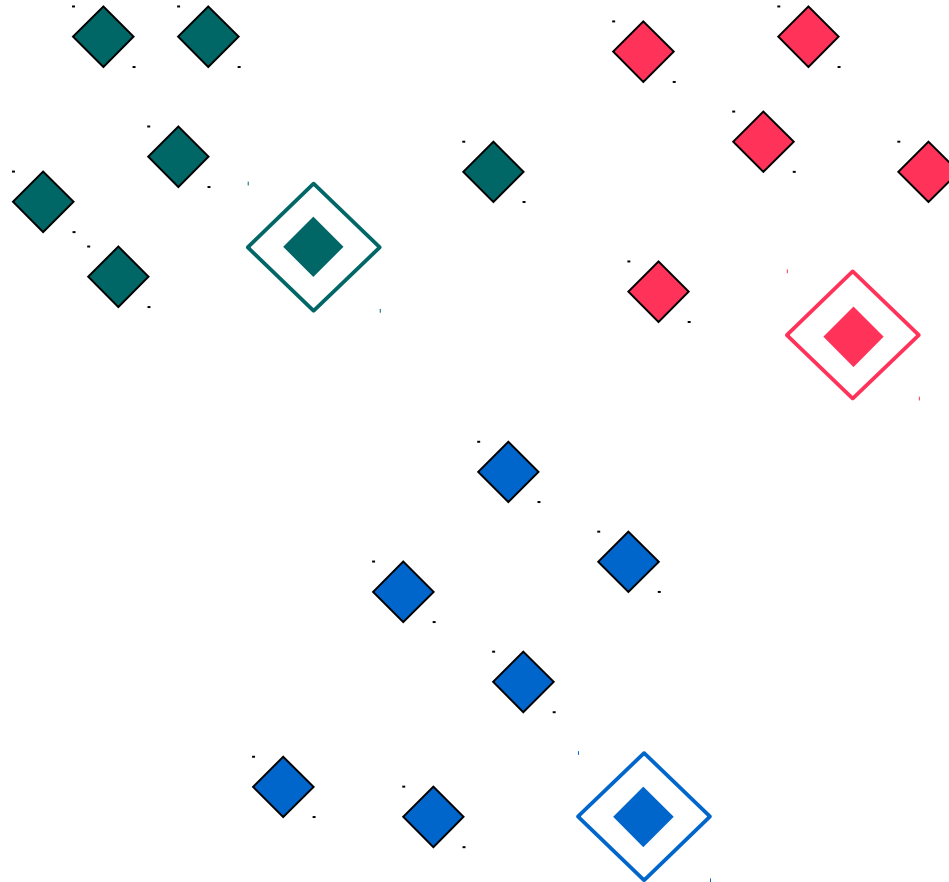
K-medoid clustering method



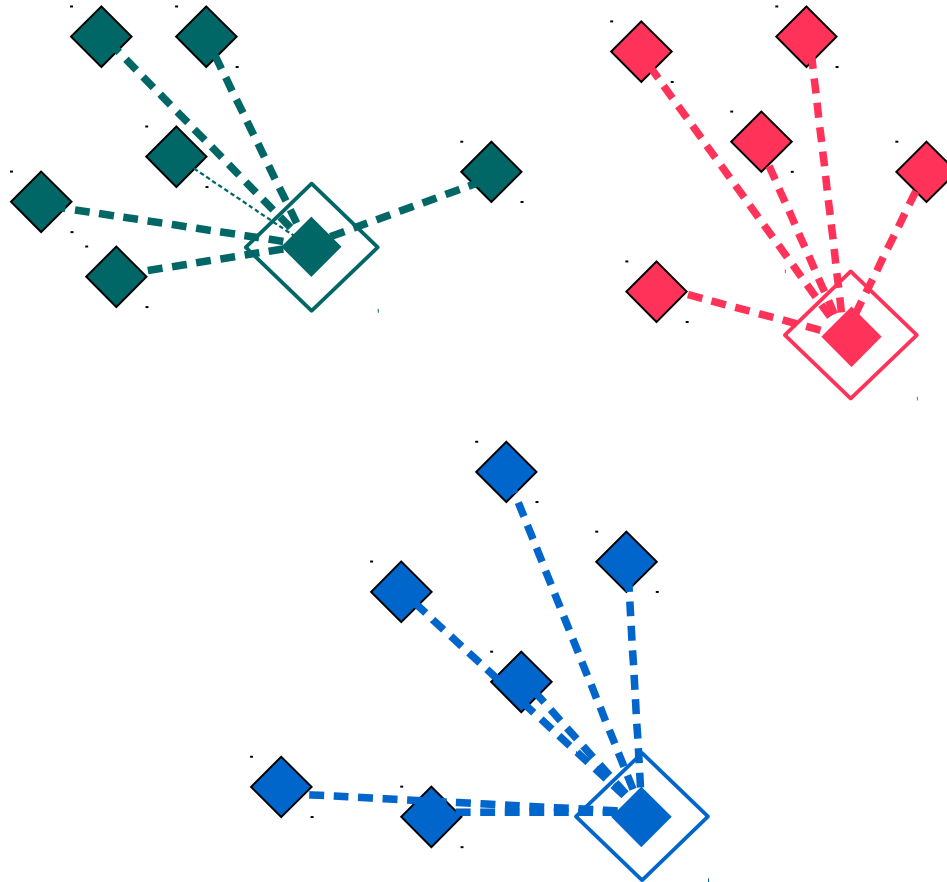
K-medoid: choose k medoids



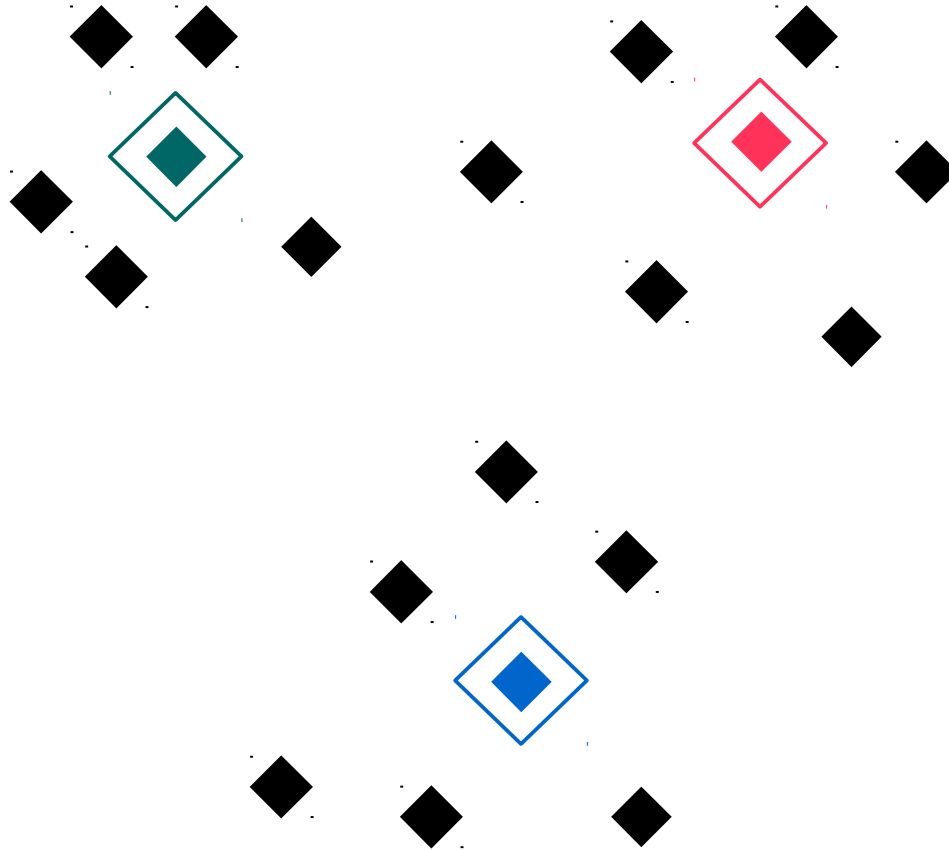
K-medoid: assign each dataset to the closest medoid



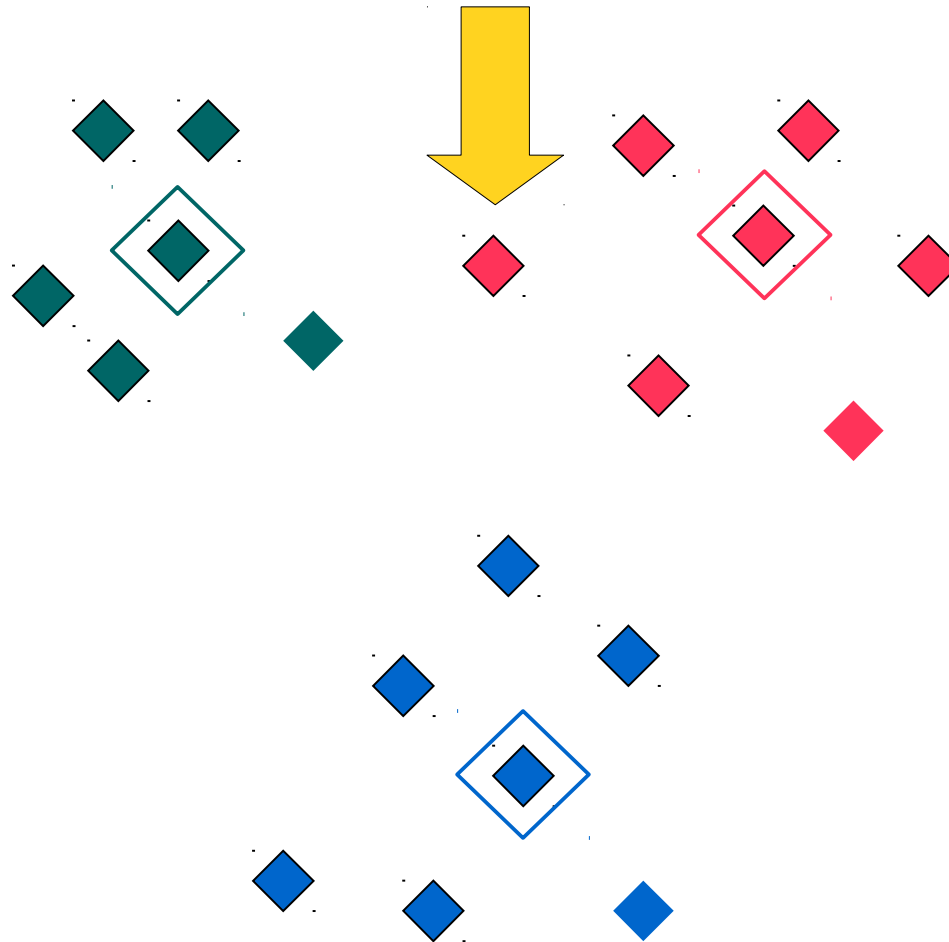
K-medoid: compute score



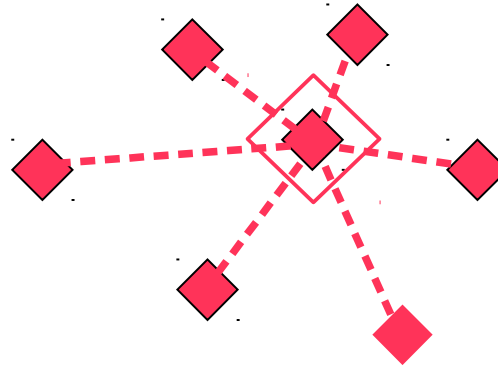
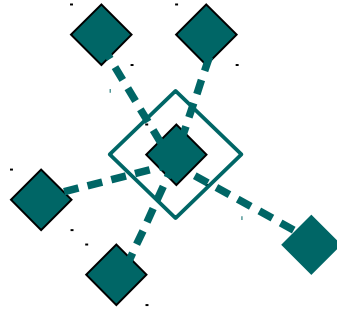
K-medoid: choose k medoids



K-medoid: assign each dataset to the new medoids



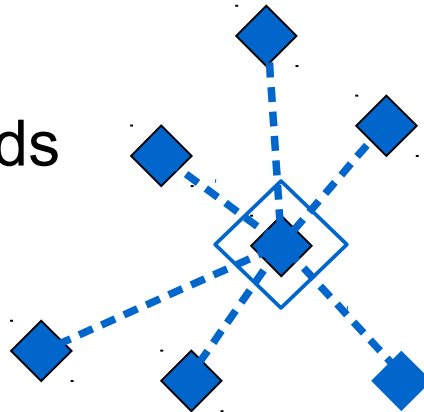
K-medoid: compute new scores



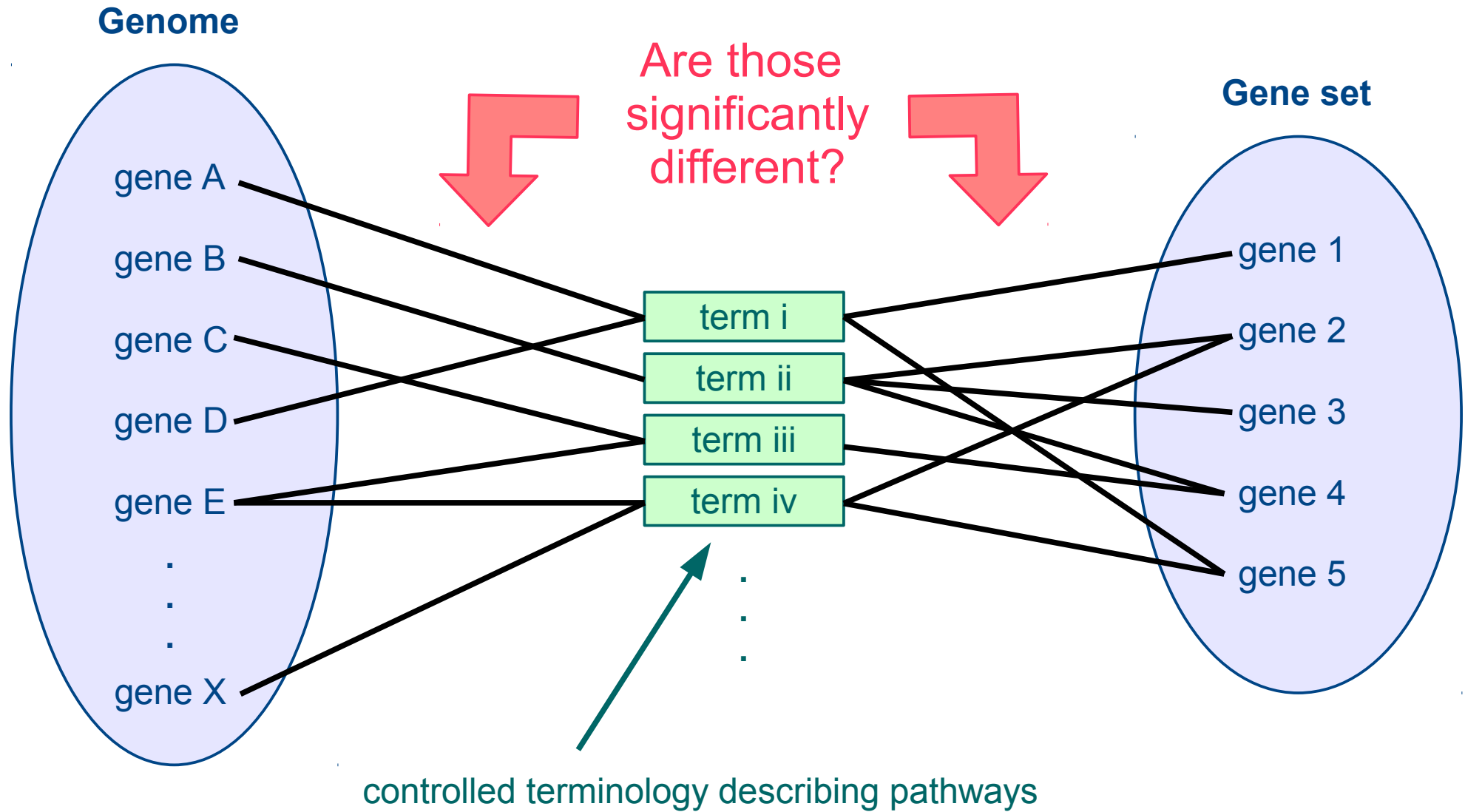
is score better?

yes → stop

no → choose new medoids



Principle of pathway enrichment



Jump To:

- biological_process**
 - behavior
 - biological adhesion
 - biological phase
 - biological regulation
 - cell aggregation
 - cell killing
 - cellular component organization or biogenesis
 - cellular process
 - developmental process
 - growth
 - immune system process
 - localization
 - locomotion
 - metabolic process
 - multi-organism process
 - multicellular organismal process
 - negative regulation of action potential
 - positive regulation of action potential
 - regulation of sequestering of zinc ion
 - reproduction
 - reproductive process
 - response to stimulus
 - rhythmic process
 - signaling
 - single-organism process
- cellular_component
- molecular_function

Phosphatidylinositol 3-kinase regulatory subunit alpha

PIK3R1

Homo sapiens (Human)

View only features (sites, domains, PTMs ...)



Reviewed - Annotation score: ●●●●● - Experimental evidence at protein level¹

BLAST

Align

Format

Add to basket

History

Function¹

Binds to activated (phosphorylated) protein-Tyr kinases, through its SH2 domain, and acts as an adaptor for glucose uptake and glycogen synthesis in insulin-sensitive tissues. Plays an important role in signaling (PubMed:17626883, PubMed:19805105, PubMed:7518429). Modulates the cellular response to ER stress overloading in the liver and hence plays a role in glucose tolerance improvement (PubMed:20348923).

GO - Molecular function¹

- 1-phosphatidylinositol-3-kinase regulator activity Source: GO_Central
- ErbB-3 class receptor binding Source: UniProtKB
- insulin binding Source: UniProtKB
- insulin-like growth factor receptor binding Source: UniProtKB
- insulin receptor binding Source: UniProtKB
- insulin receptor substrate binding Source: BHF-UCL
- neurotrophin TRKA receptor binding Source: UniProtKB
- phosphatidylinositol 3-kinase binding Source: BHF-UCL
- phosphatidylinositol 3-kinase regulator activity Source: UniProtKB
- protein phosphatase binding Source: UniProtKB
- transcription factor binding Source: UniProtKB
- transmembrane receptor protein tyrosine kinase adaptor activity Source: BHF-UCL

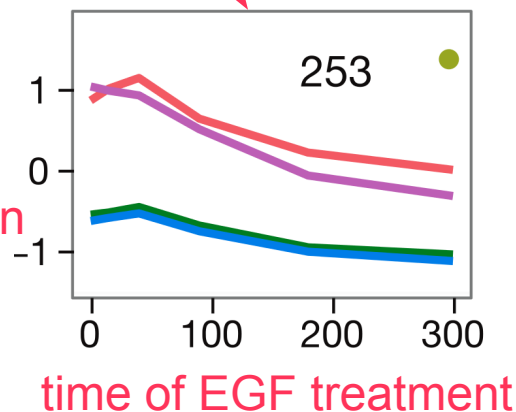
GO - Biological process¹

- B cell differentiation Source: Ensembl
- blood coagulation Source: Reactome
- cellular glucose homeostasis Source: UniProtKB
- cellular response to insulin stimulus Source: UniProtKB
- cellular response to UV Source: Ensembl
- epidermal growth factor receptor signaling pathway Source: Reactome
- extrinsic apoptotic signaling pathway via death domain receptors Source: Ensembl
- Fc-epsilon receptor signaling pathway Source: Reactome

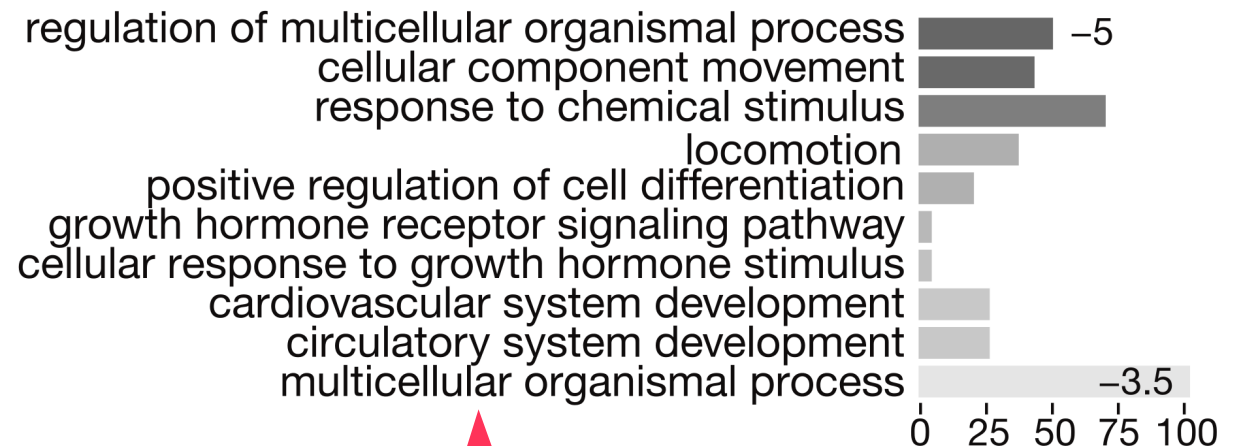
What am I showing you on the next slides?

one cluster,
comprising 253 genes

centred
normal
expression



log p-value
(darker = more significant)



Gene expression profiles in MCF10A cells upon EGF stimulation

Gene HGNC symbol / Ensembl ID

LBH

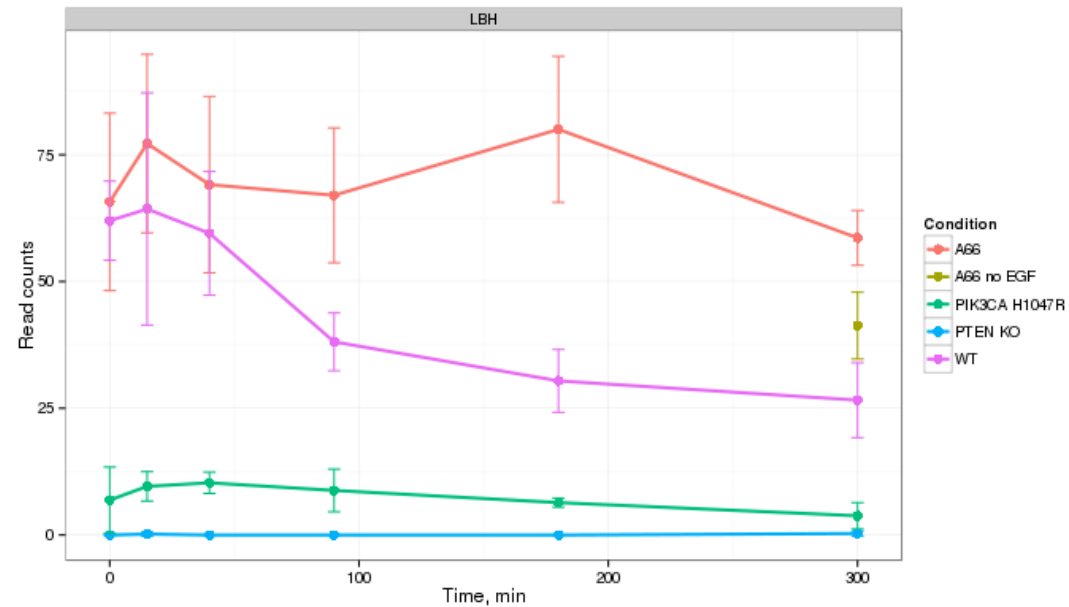
☐ RPKM

Please click on links below only after the plot appears on the right.

[Download Plot](#)

[Download Data](#)

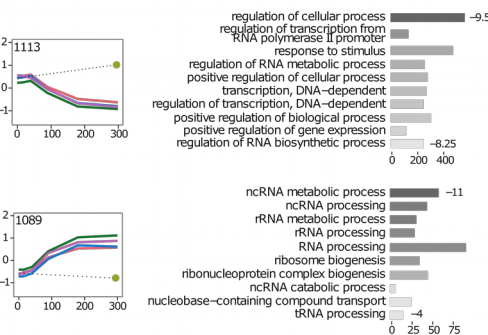
This is a Supplementary figure for the paper...
(read counts are normalized by library sizes)



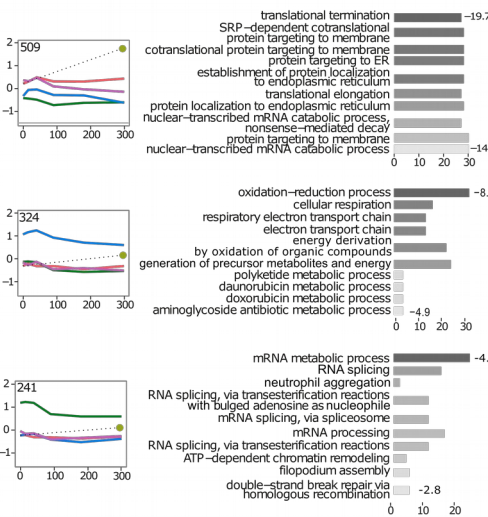
EGF

PI3K

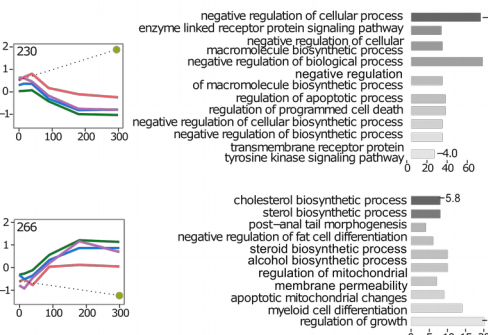
1
Regulation of transcription
Ribosome biogenesis
ncRNA/ rRNA/ RNA processing



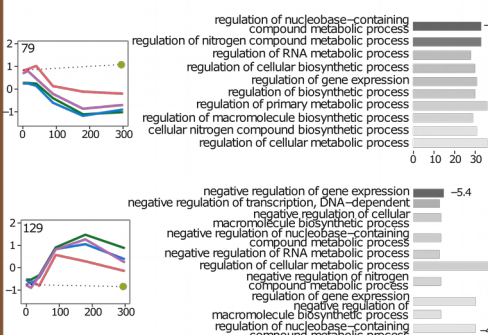
2
Cellular respiration/ Energy derivation
mRNA processing and splicing
Translation elongation/ termination



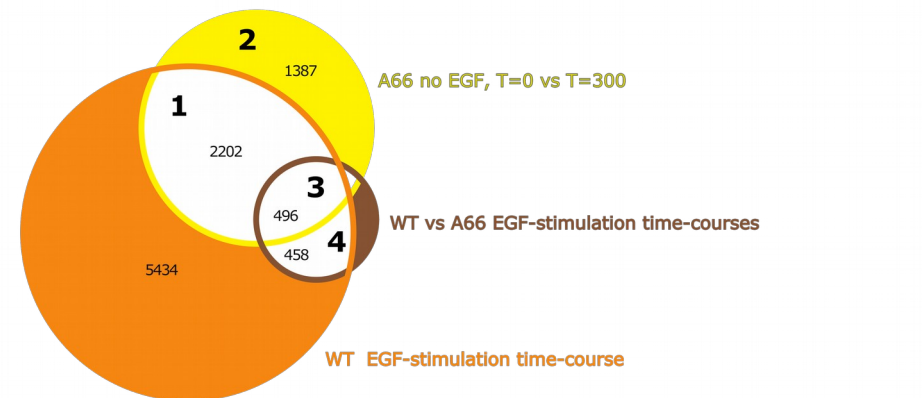
3
Apoptosis
Cell death
Sterol metabolism



4
Regulation of RNA metabolism
Regulation of gene expression

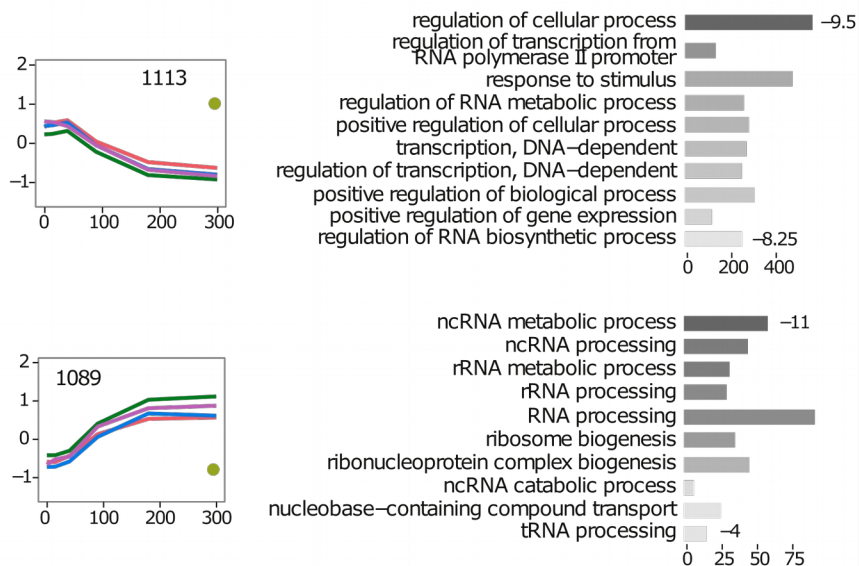


- WT
- A66
- A66 no EGF
- PTEN KO
- PIK3CA H1047R



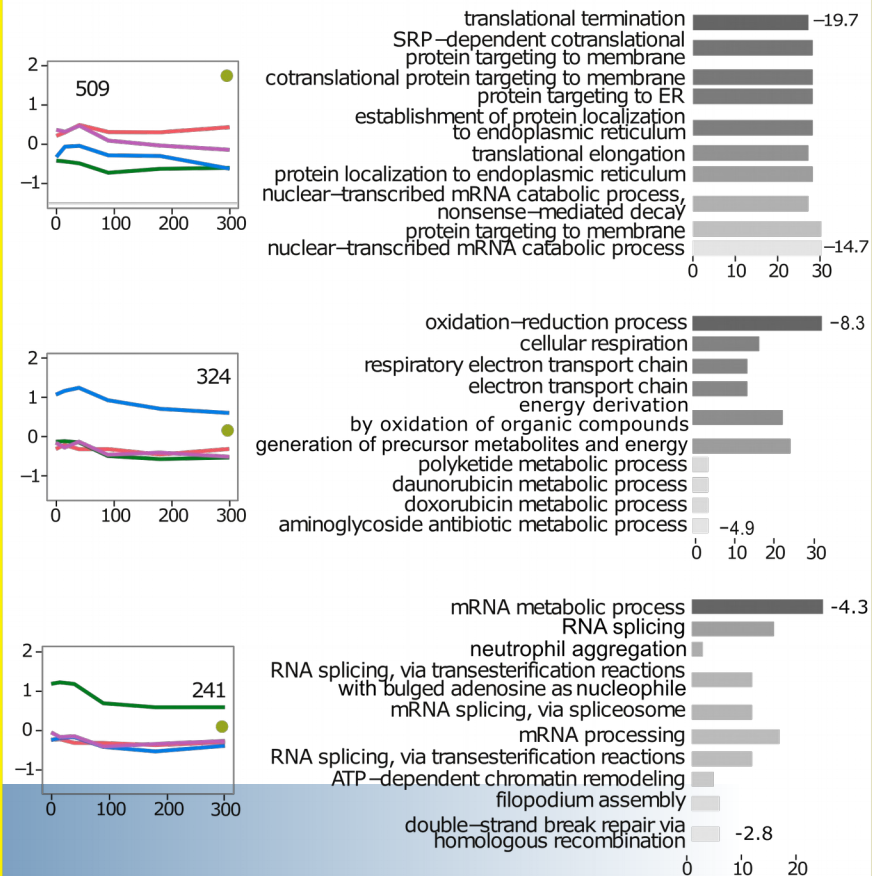
1

Regulation of transcription Ribosome biogenesis ncRNA/ rRNA/ RNA processing



2

Cellular respiration/ Energy derivation mRNA processing and splicing Translation elongation/ termination

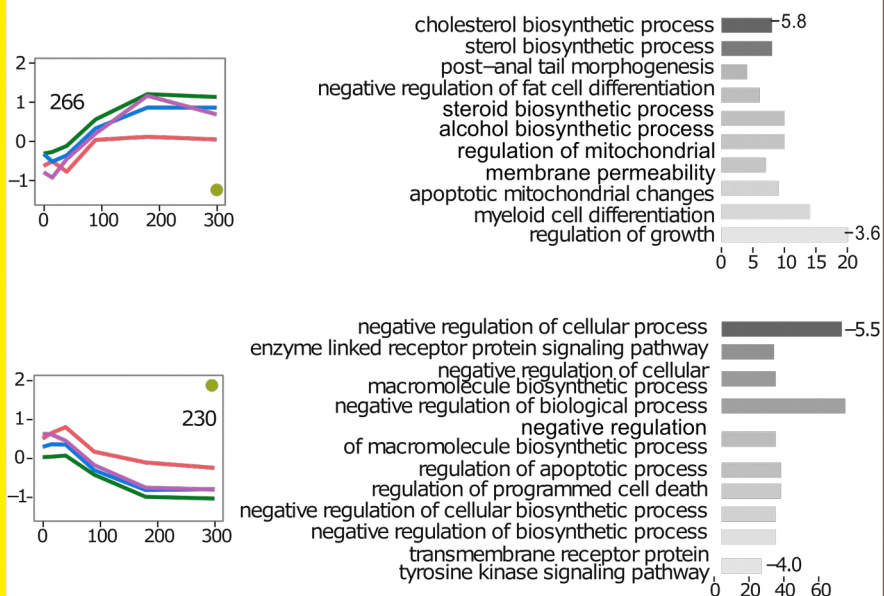


GF

3K

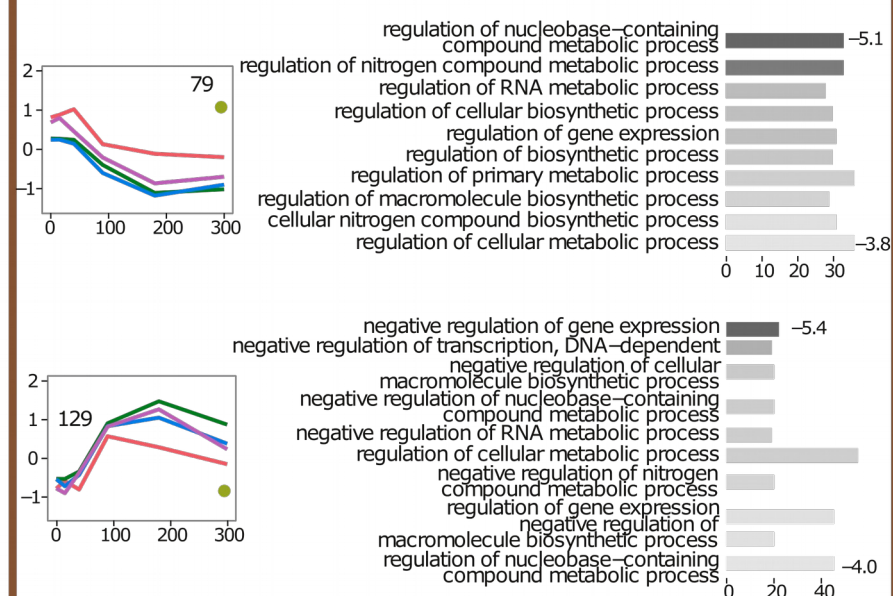
3

Apoptosis Cell death Sterol metabolism



4

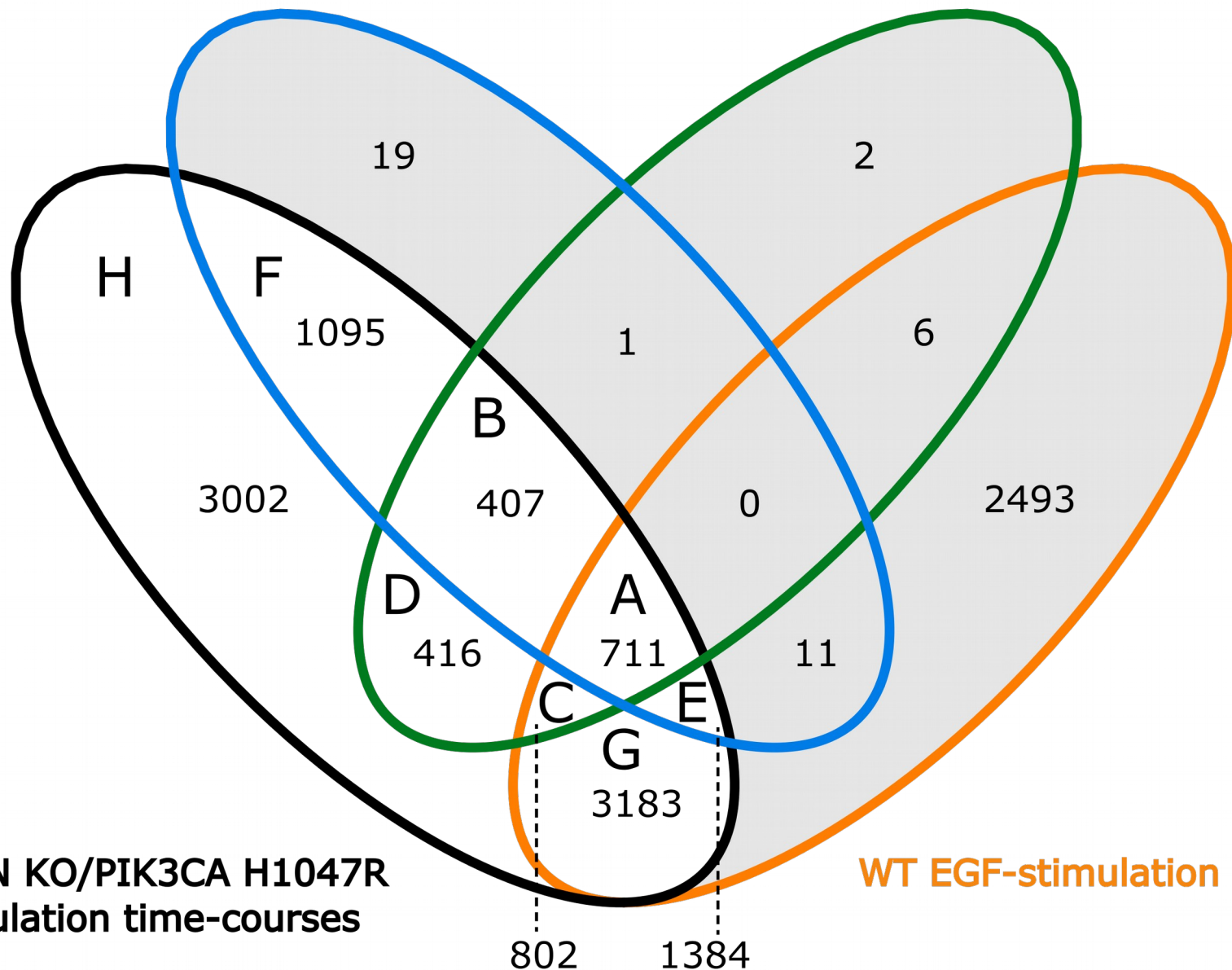
Regulation of RNA metabolism Regulation of gene expression



Effects of constitutive mutations

WT vs PTEN KO at T=0

WT vs PIK3CA H1047R at T=0



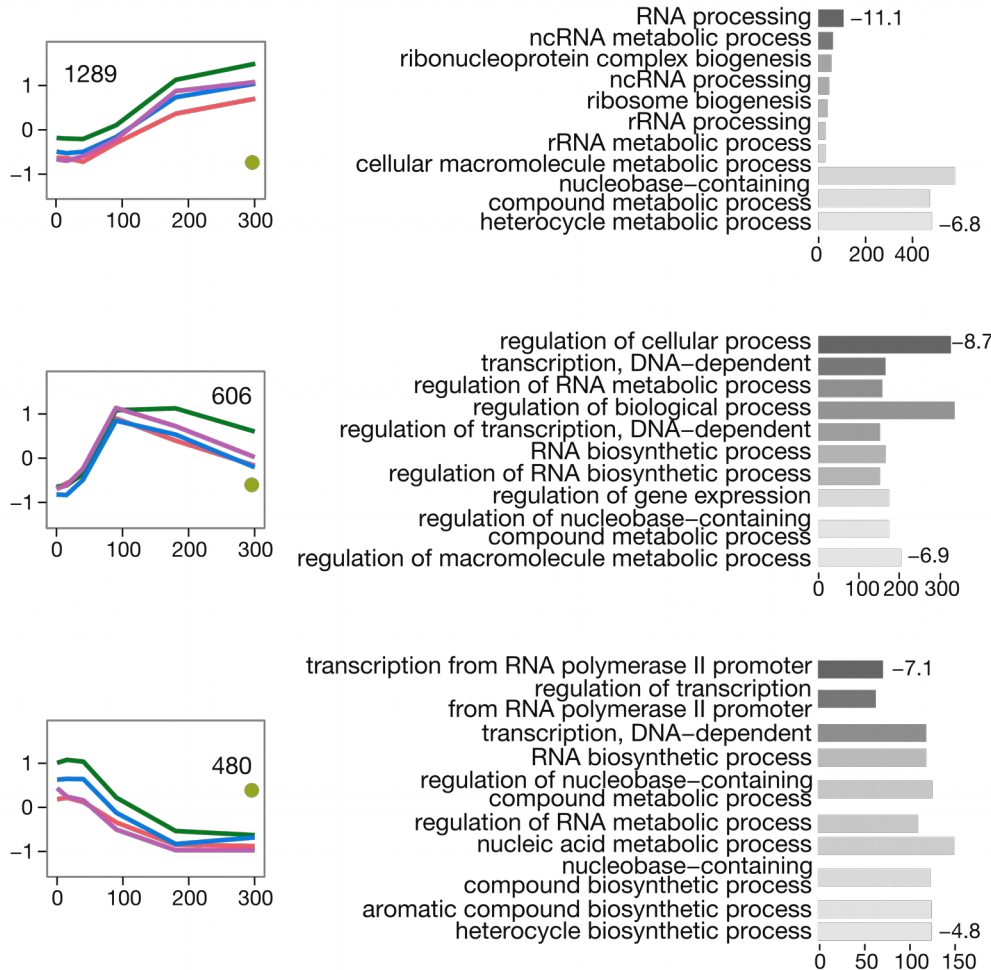
WT vs PTEN KO/PIK3CA H1047R
EGF-stimulation time-courses

WT EGF-stimulation time-course

PIK3CA H1047R + PTEN KO

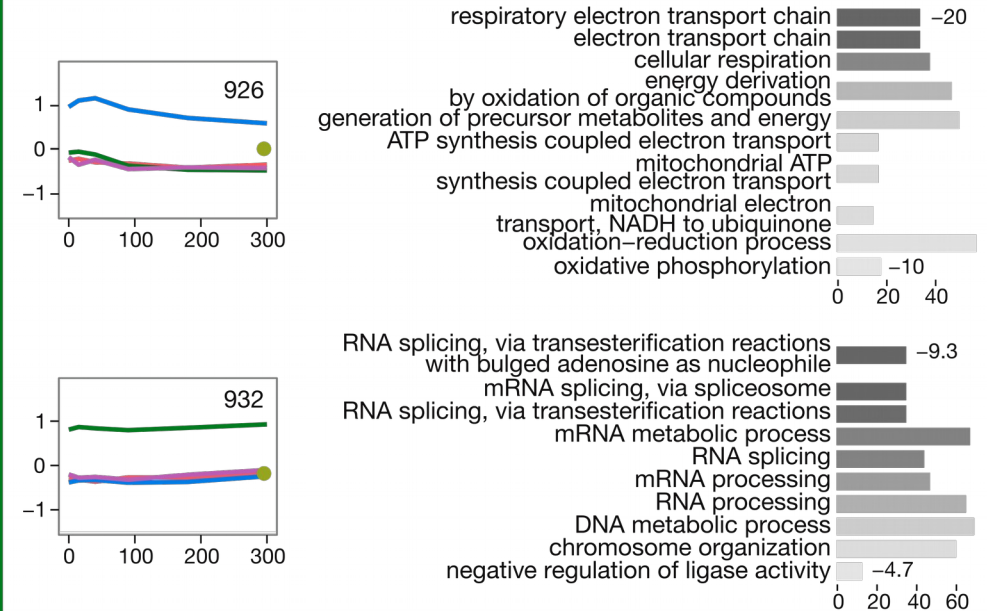
G

ncRNA/ rRNA/ RNA processing Ribosome biogenesis Transcription



H

Cell respiration Energy derivation mRNA splicing/ processing

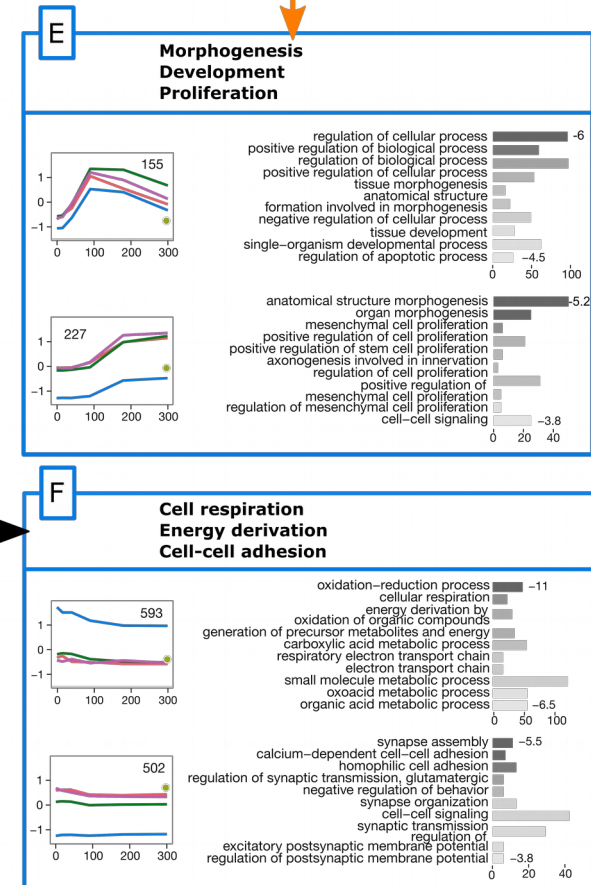
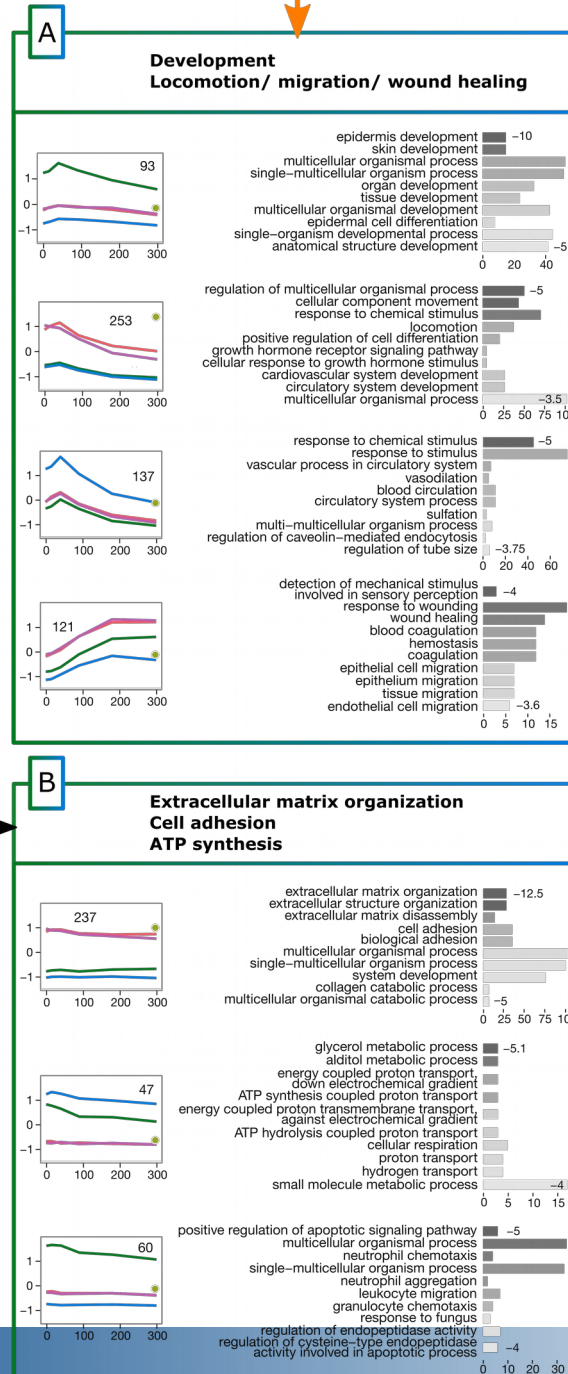
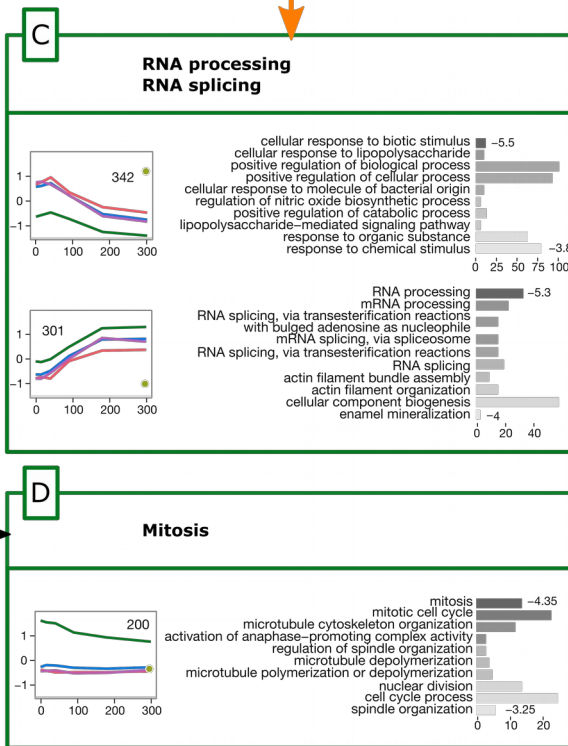


PI3K responding to EGF
in mutants
(~ groups 1 and 2 of A66)

PIK3CA H1047R

PIK3CA H1047R + PTEN KO

PTEN KO



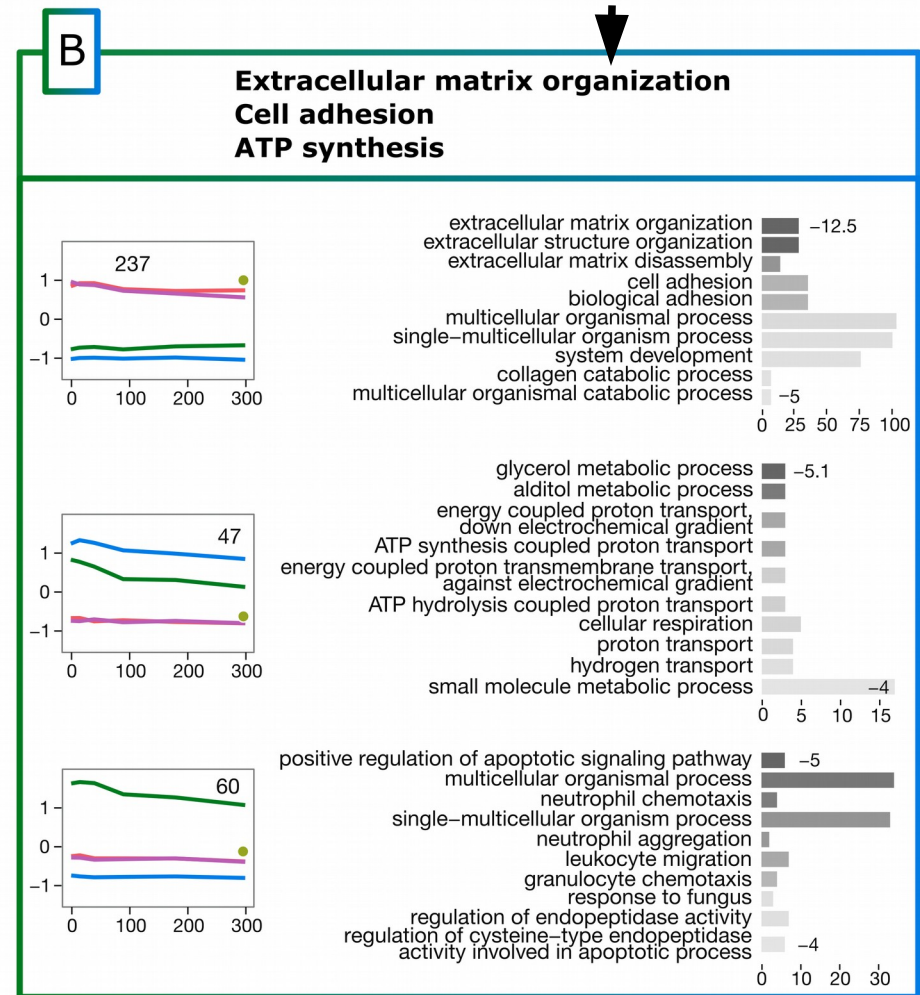
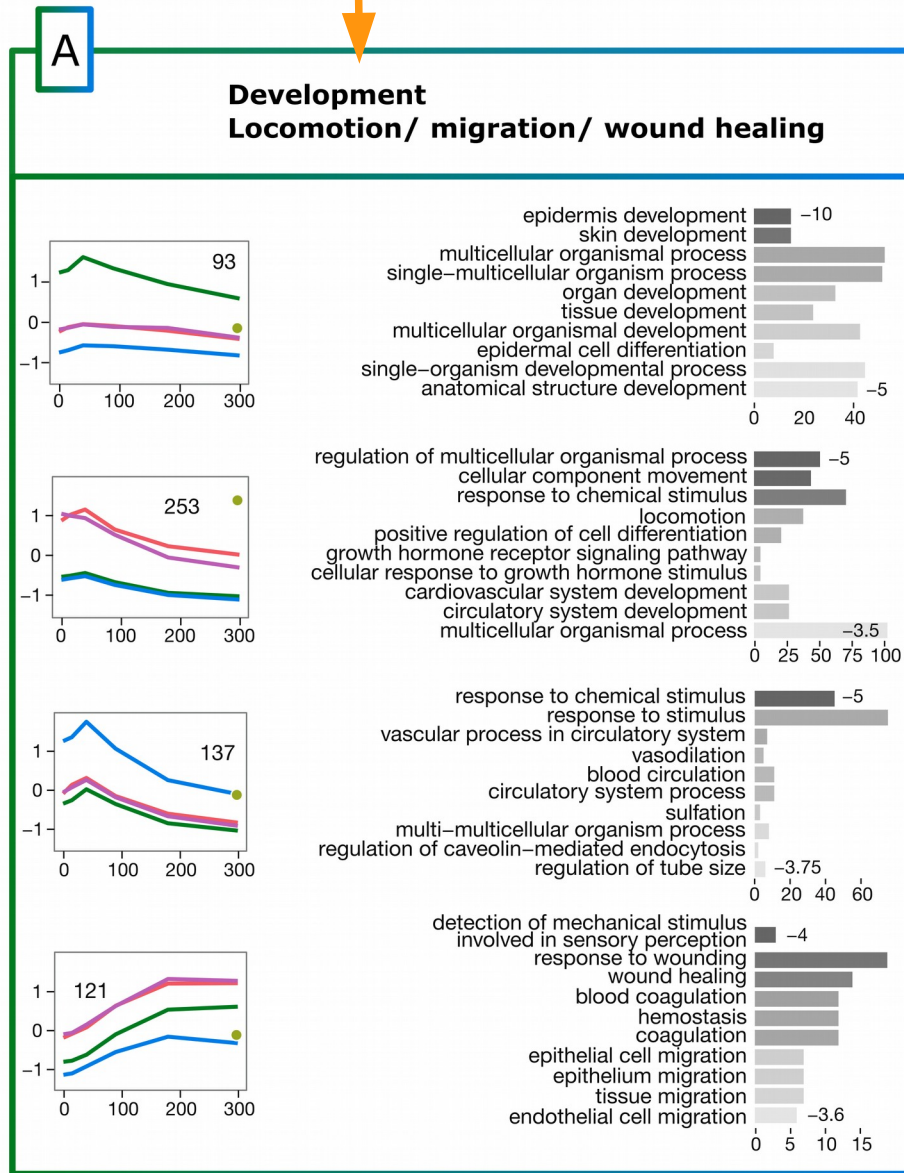
Genes affected by the mutations even without EGF perturbations



responding
to EGF in WT

PIK3CA H1047R + PTEN KO

no response
to EGF in WT



Genes responding to both mutations
in a coherent fashion; affected
by EGF in the mutants

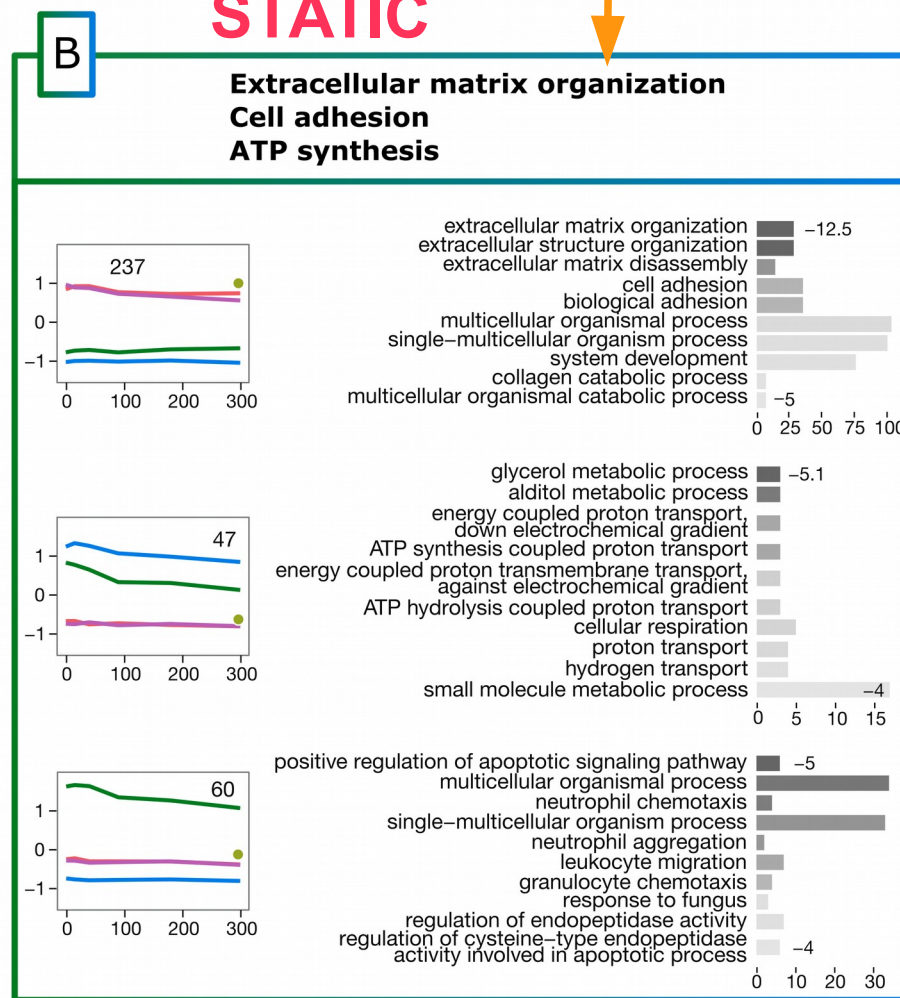
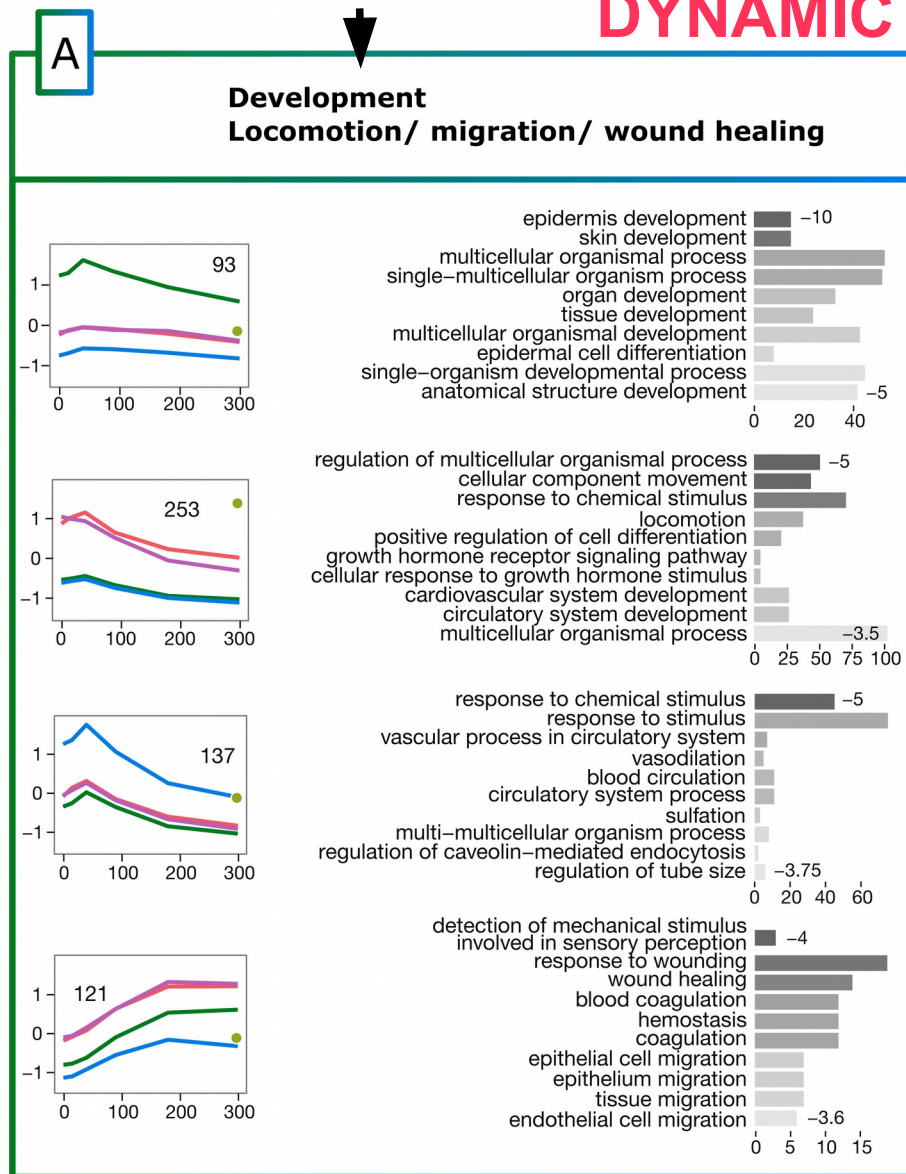
no response
to EGF in WT

PIK3CA H1047R + PTEN KO

responding
to EGF in WT

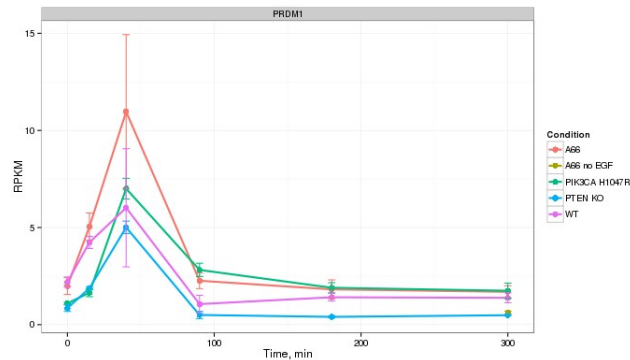
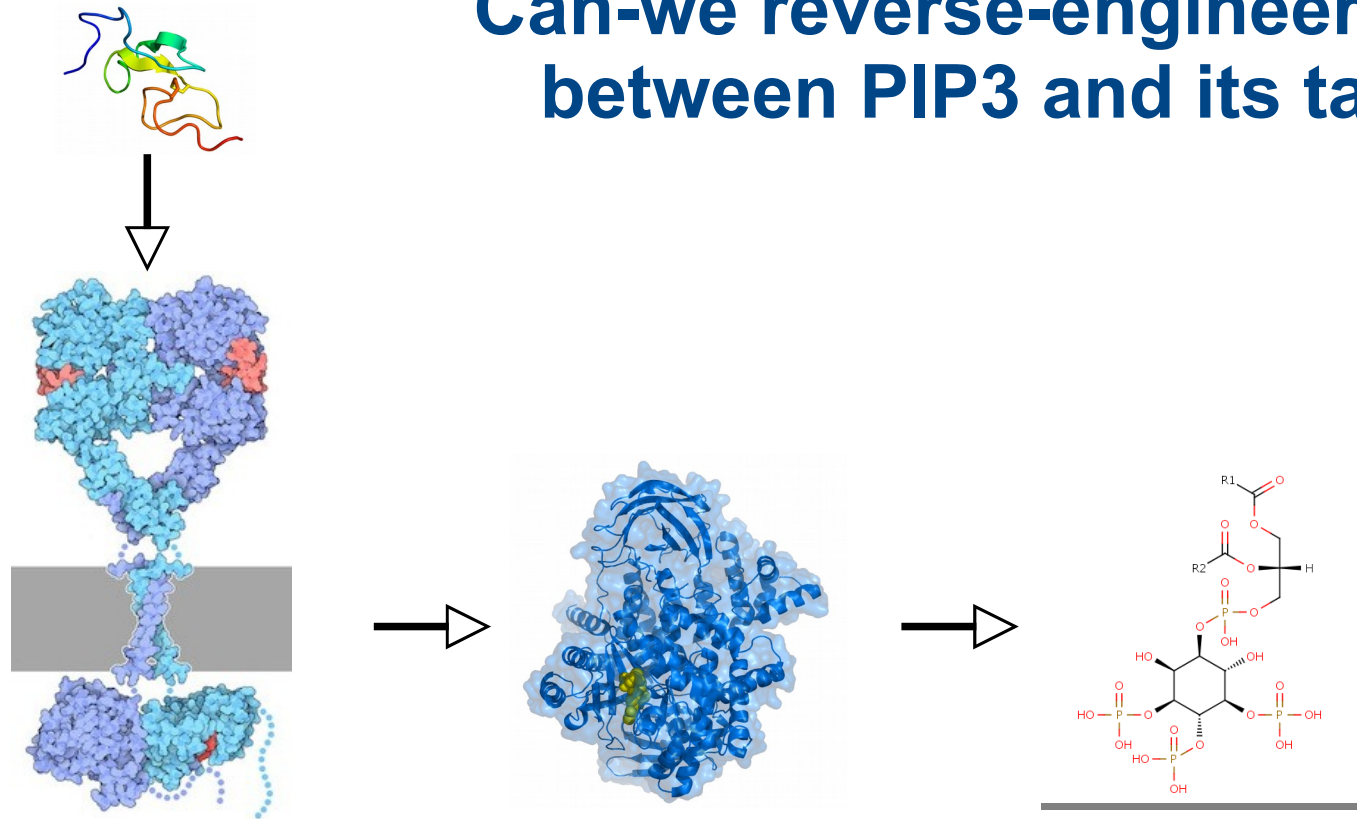
DYNAMIC

STATIC



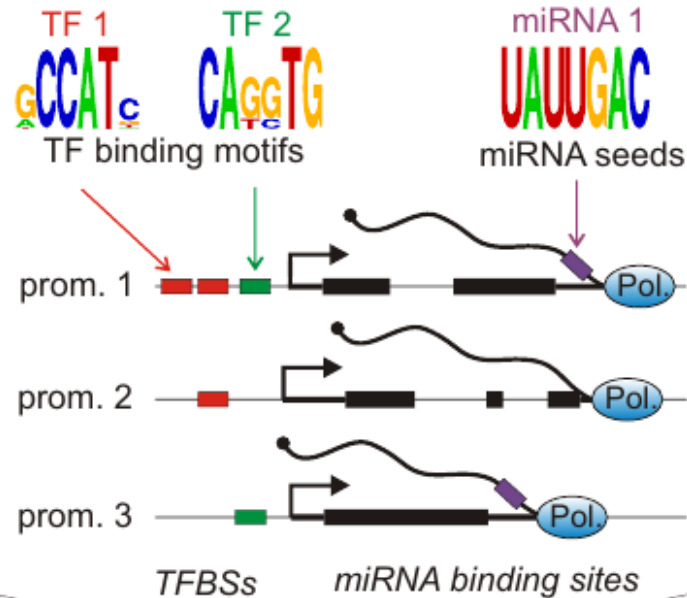
Genes responding to both mutations
in a coherent fashion

Can we reverse-engineer the link between PIP3 and its targets?

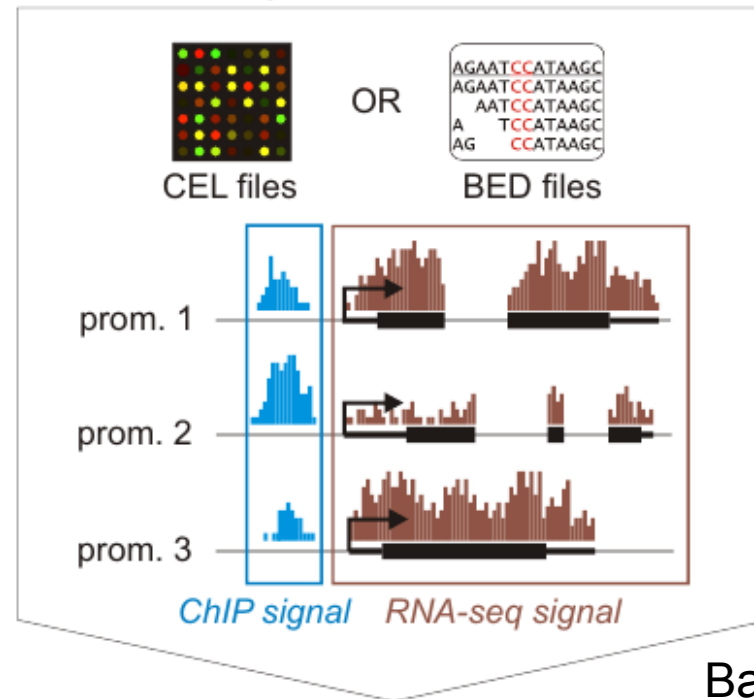


TF?

A) identification of regulatory sites



B) measurement



C) normalization and summation

$$N_{pm} = \begin{matrix} & \text{red} & \text{green} & \text{purple} \\ \text{prom. 1} & 2 & 1 & 1 & \dots \\ \text{prom. 2} & 1 & 0 & 0 & \dots \\ \text{prom. 3} & 0 & 1 & 1 & \dots \\ & \vdots & \vdots & \vdots & \ddots \end{matrix}$$

TF & miRNA binding site count

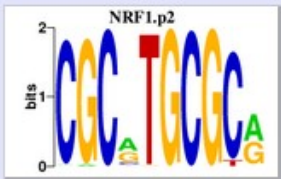
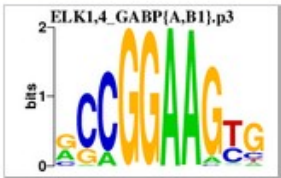
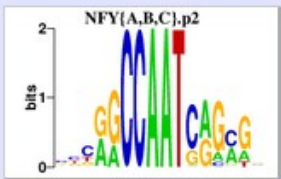
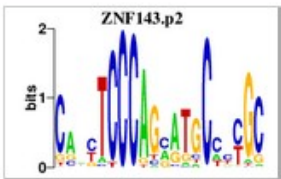
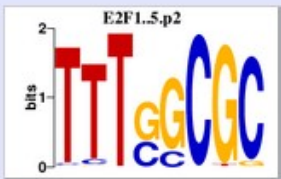
$$E_{ps} = \begin{matrix} & \text{samples} \\ \text{prom. 1} & \dots \\ \text{prom. 2} & \dots \\ \text{prom. 3} & \dots \end{matrix}$$

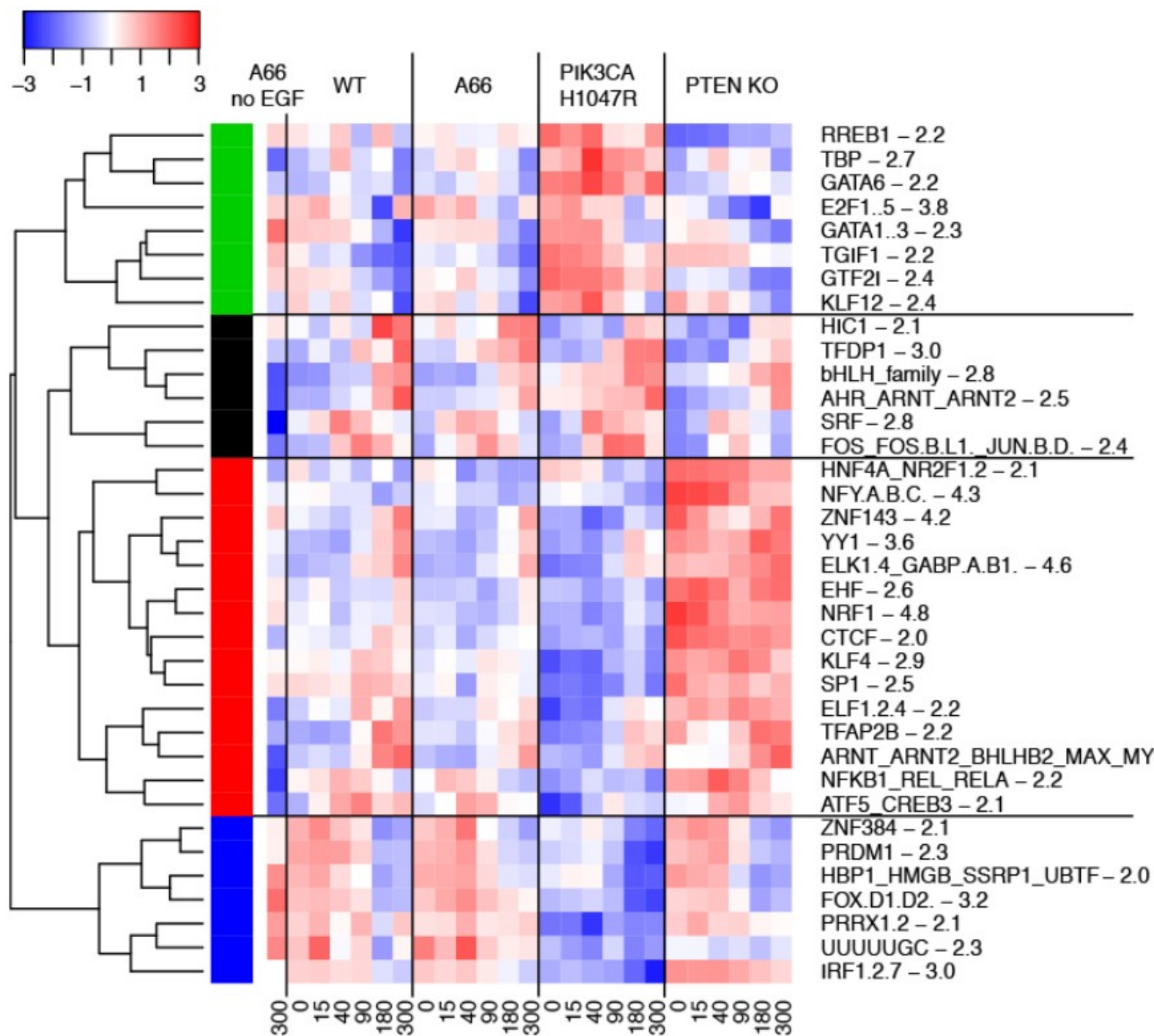
expression or epigenetic signal level

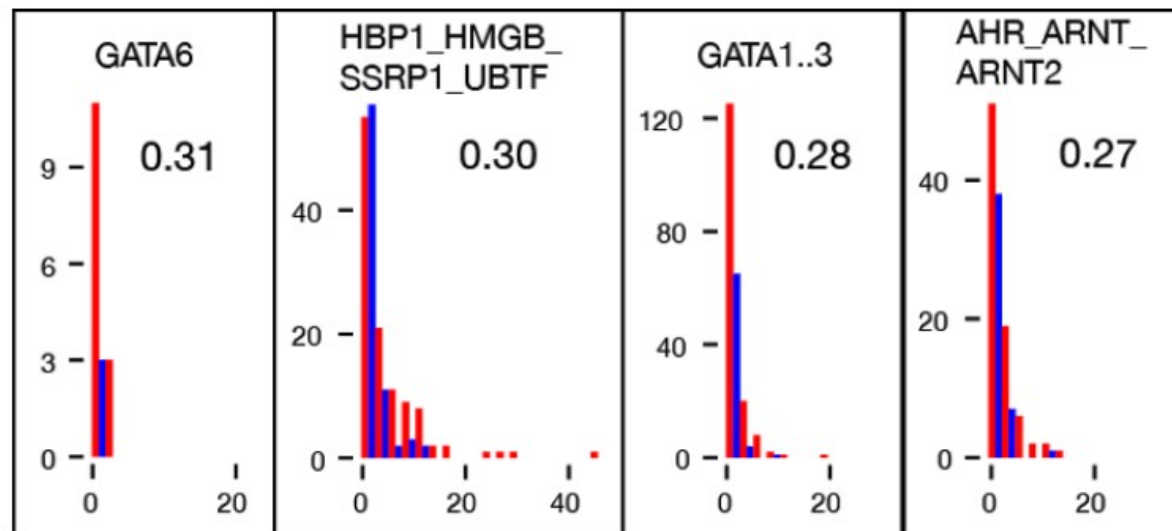
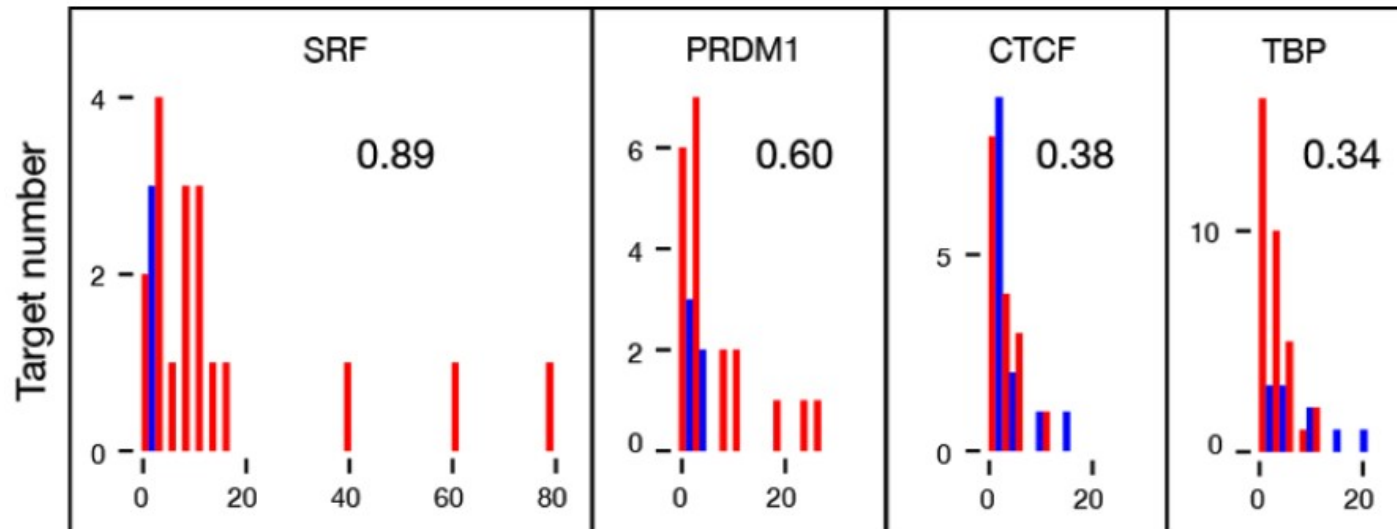
D) MARA model

$$E_{ps} = \sum_m N_{pm} \cdot A_{ms} + c_p + \tilde{c}_s$$

Balwierz et al (2014)
Genome Res

Motif name	Z-value	Associated genes	Profile	Logo
NRF1.p2	4.755	NRF1 (EWG, ALPHA-PAL)		
ELK1.4_GABP{A,B1}.p3	4.633	GABPA (E4TF1A, NFT2, NRF2, E4TF1-60, NRF2A) GABPB1 (E4TF1-47, GABPB) ELK4 (SAP1) ELK1		
NFY{A,B,C}.p2	4.292	NFYC (CBF-C) NFYB (CBF-A, HAP3) NFYA (HAP2, CBF-B)		
ZNF143.p2	4.154	ZNF143 (SBF, pHZ-1, STAF)		
E2F1..5.p2	3.631	E2F4 (E2F-4) E2F5 E2F2 (E2F-2) E2F1 (RBP3) E2F3		
				



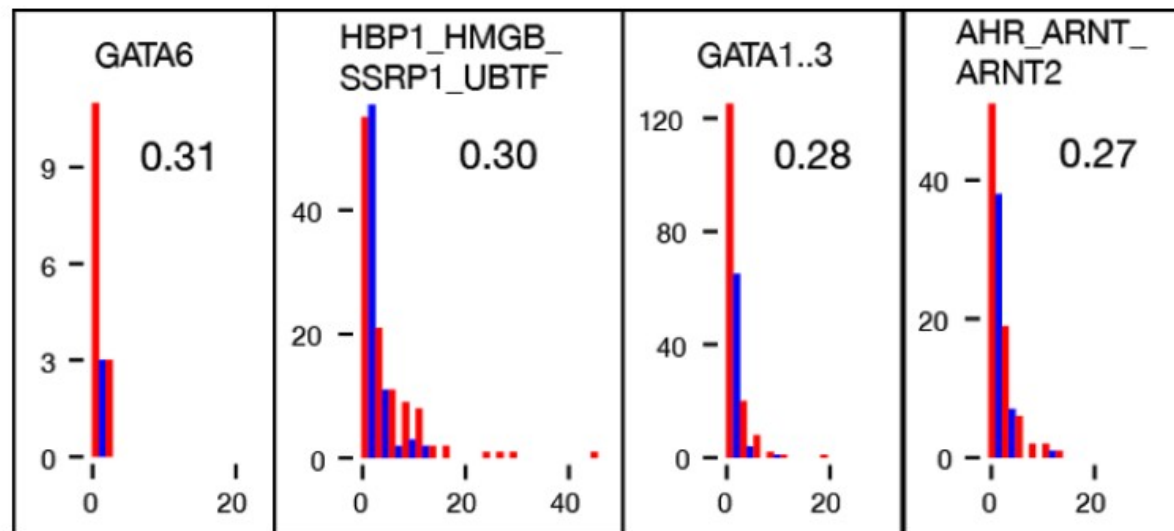
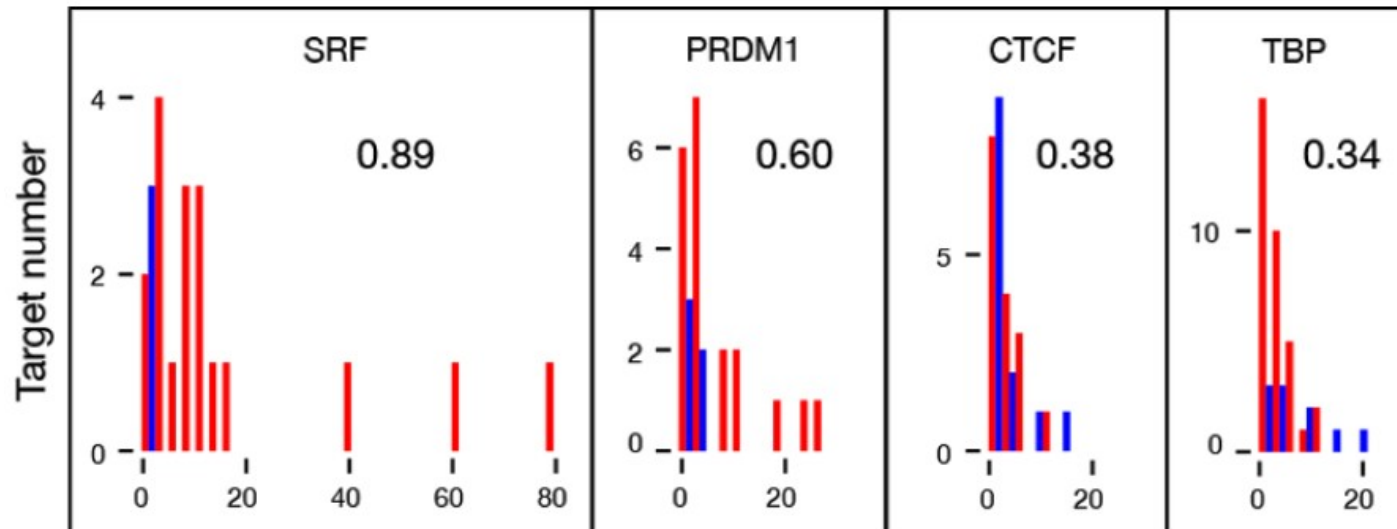


expression

■ A (EGF sens.)
■ B (EGF insens.)

known

new



expression

■ A (EGF sens.)
■ B (EGF insens.)

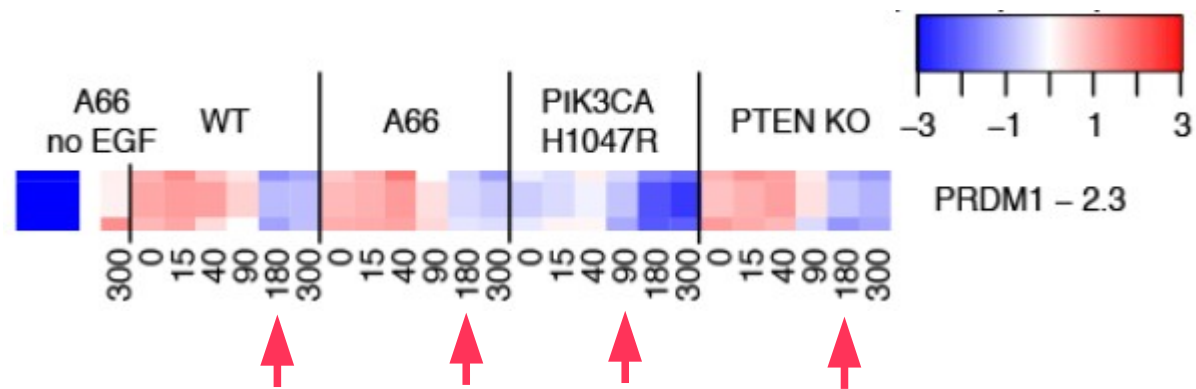
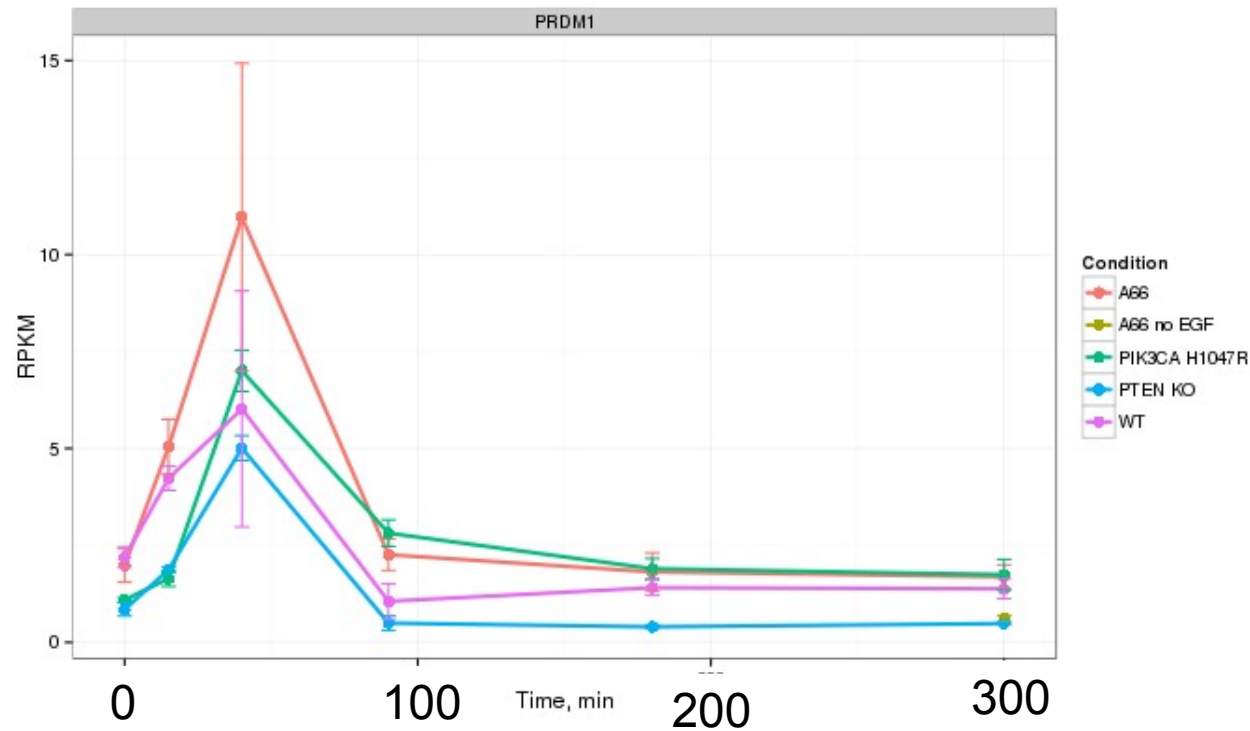
BLIMP1 (PRDM1) targets

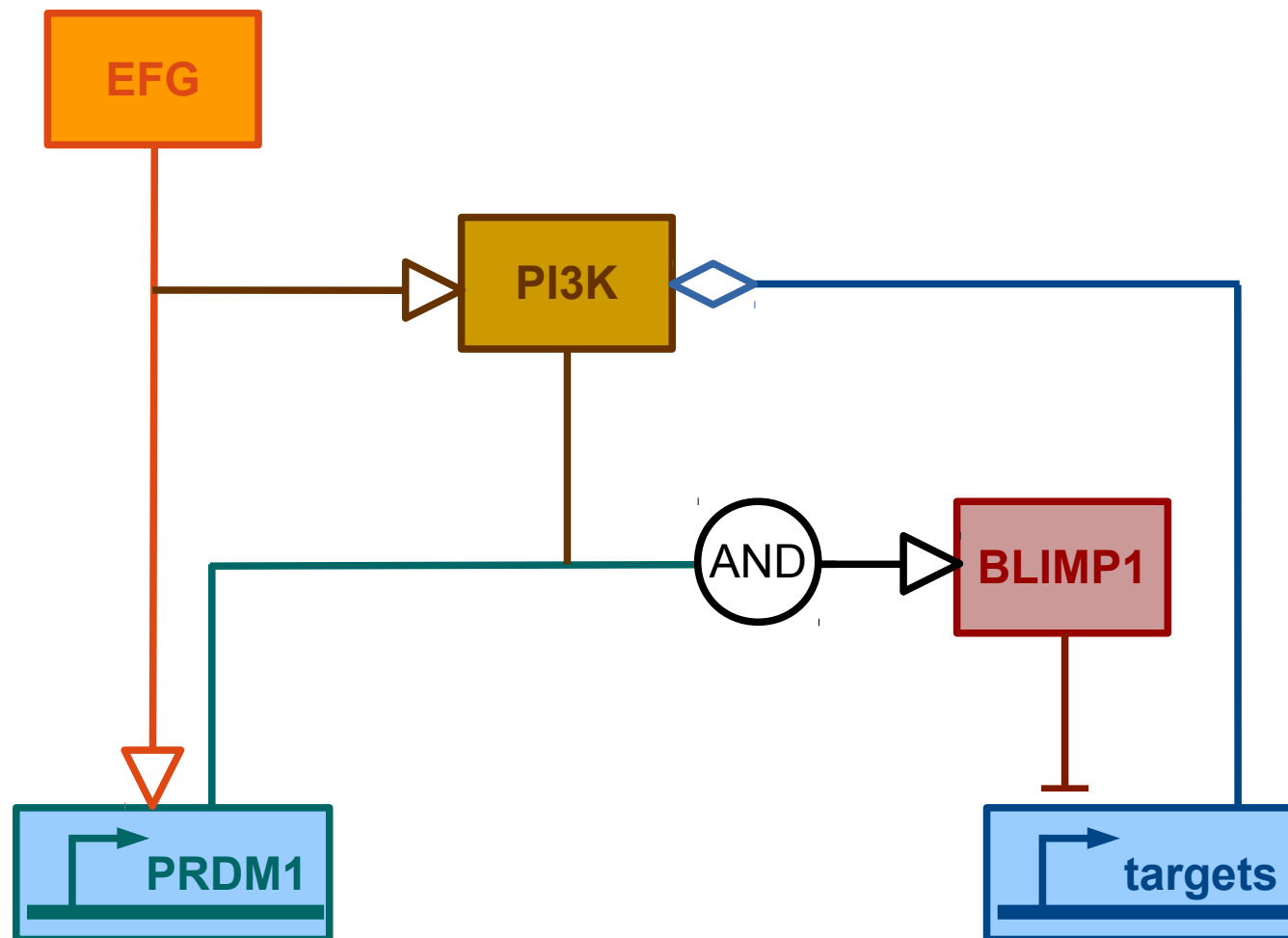
GLUL	4.56994		
NEDD4L	4.42406		Possible inhibition of PI3K phosphorylation (Kovacevic et al. 2013)
VWA5A	2.90631		Breast tumor suppressor
PPP1R3B	2.76543		
CIR1	1.69659		
ZNF737	1.37612		
LPXN	1.20711		Interacts with tyrosine kinases (upstream of PI3K)
FAM134B	1.06833		
GAB2	0.844677		Involved in the activation of PI3K (Gu et al. 2000)
EPHX1	0.657508		

Motif target gene	Sm	Gene profile	Description
KCNB1	28.0333		
NCOA7	23.8204		
LIF	20.1823		
ASPA	11.6217		
SORBS2	11.2664		
UBA7	9.8144		
LMCD1	9.23465		
CYLD	5.01778		Deubiquitination of AKT (Lim et al. 2012)
TAPBPL	4.94158		
PIK3IP1	4.6666		Negative regulator of hepatic PI3K activity (He et al. 2008)

EGF → PRDM1 expression

PIP3 → BLIMP1 function





Summary

- 1) Most effects of EGF on gene expressions are not mediated by PIP3
(not surprising)
- 2) Expression of a very large number of genes is affected by PIP3 perturbations: “Butterfly effect”
- 3) Different perturbations affect different gene populations
- 4) Subset of coherent effects: “static” cellular functions are EGF-insensitive, while “dynamic” are EGF-sensitive
- 5) Blimp1 is identified as a new TF downstream of PIP3
- 6) Blimp1 targets form a transcriptional feedback loop on PIP3 signalling



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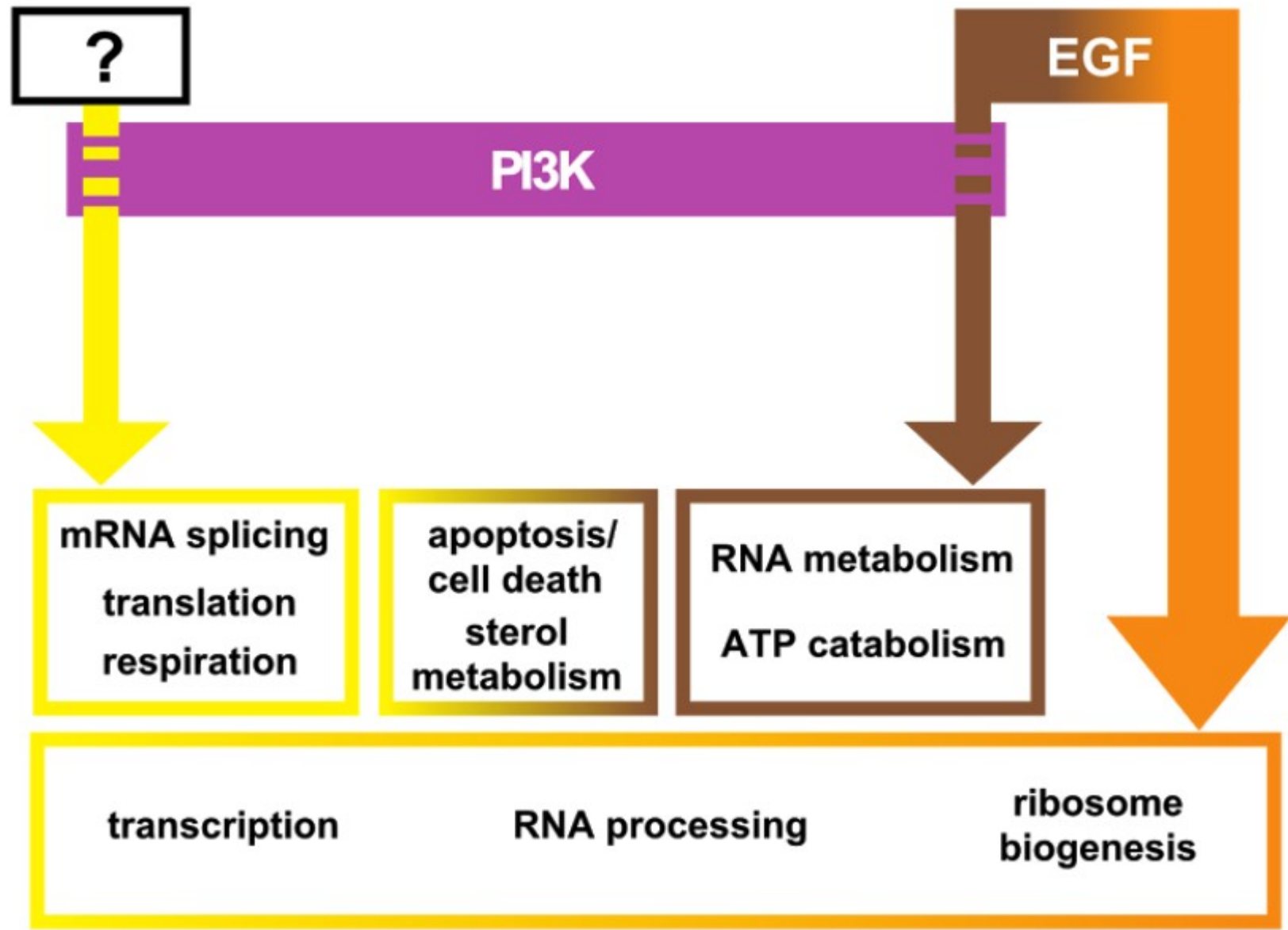
Bioinformatics team

Simon Andrews

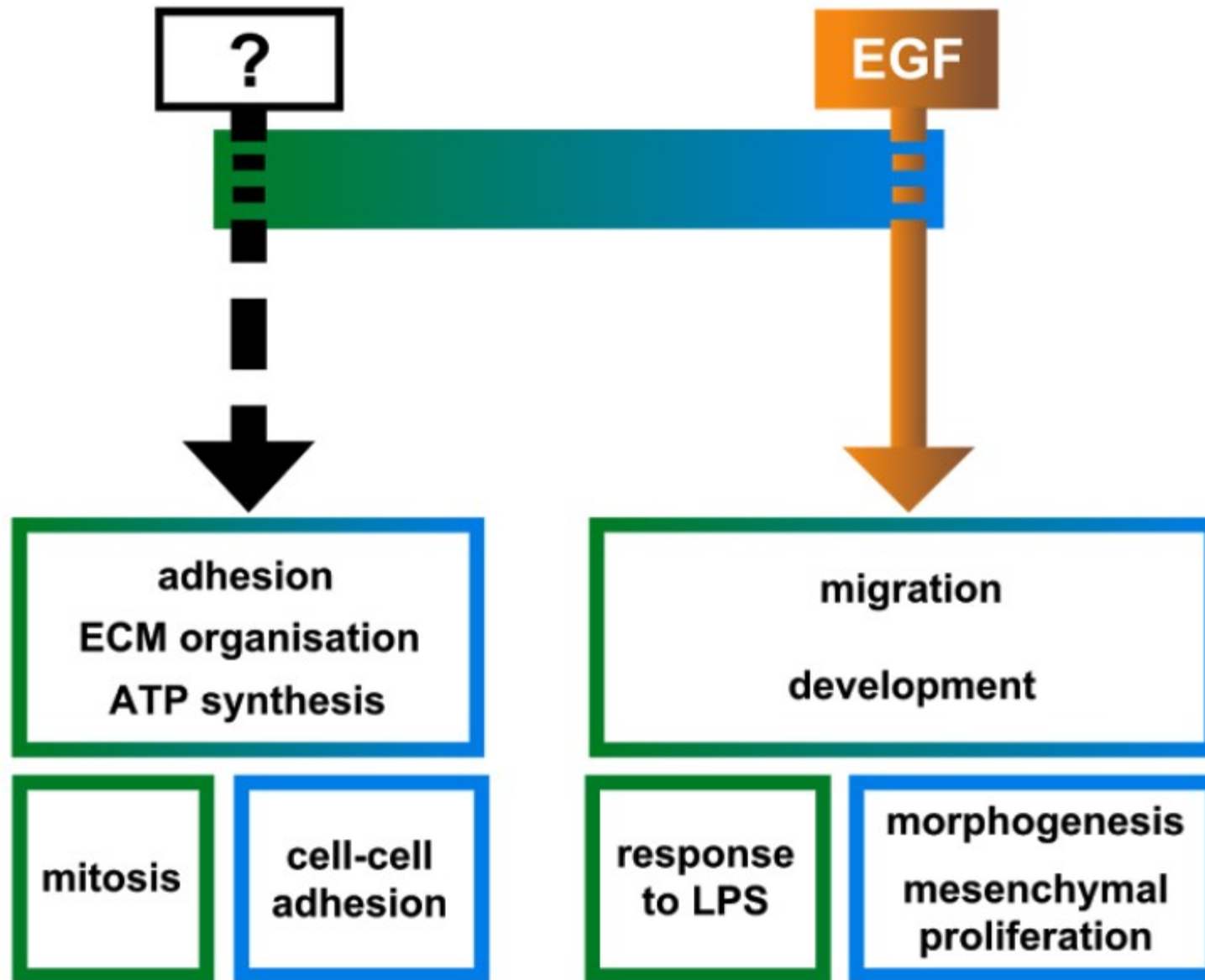
Anne Segond-Pichon



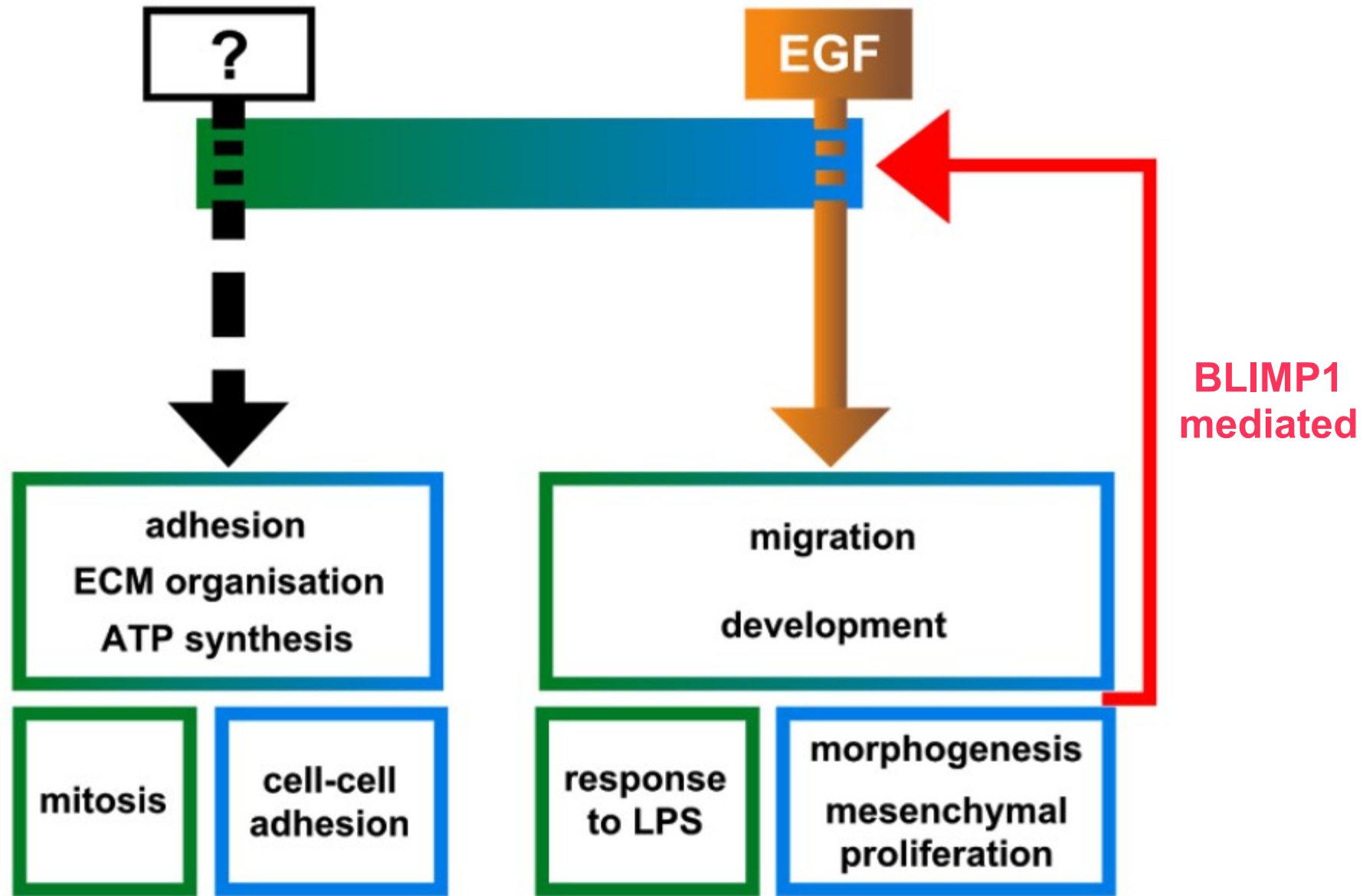
Acute perturbation of PIP3 signalling



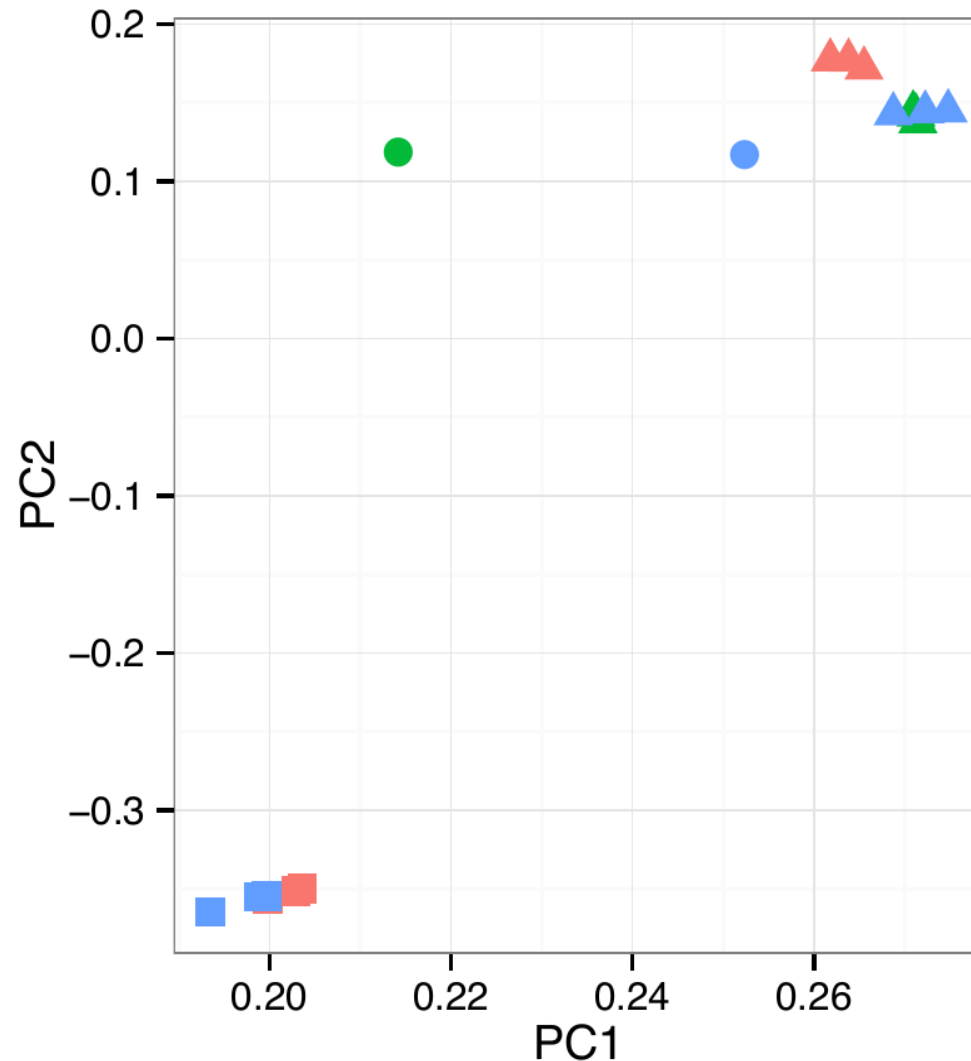
Chronic perturbation of PIP3 signalling



Chronic perturbation of PIP3 signalling



Biggest source of variability is the lab ...



Paper

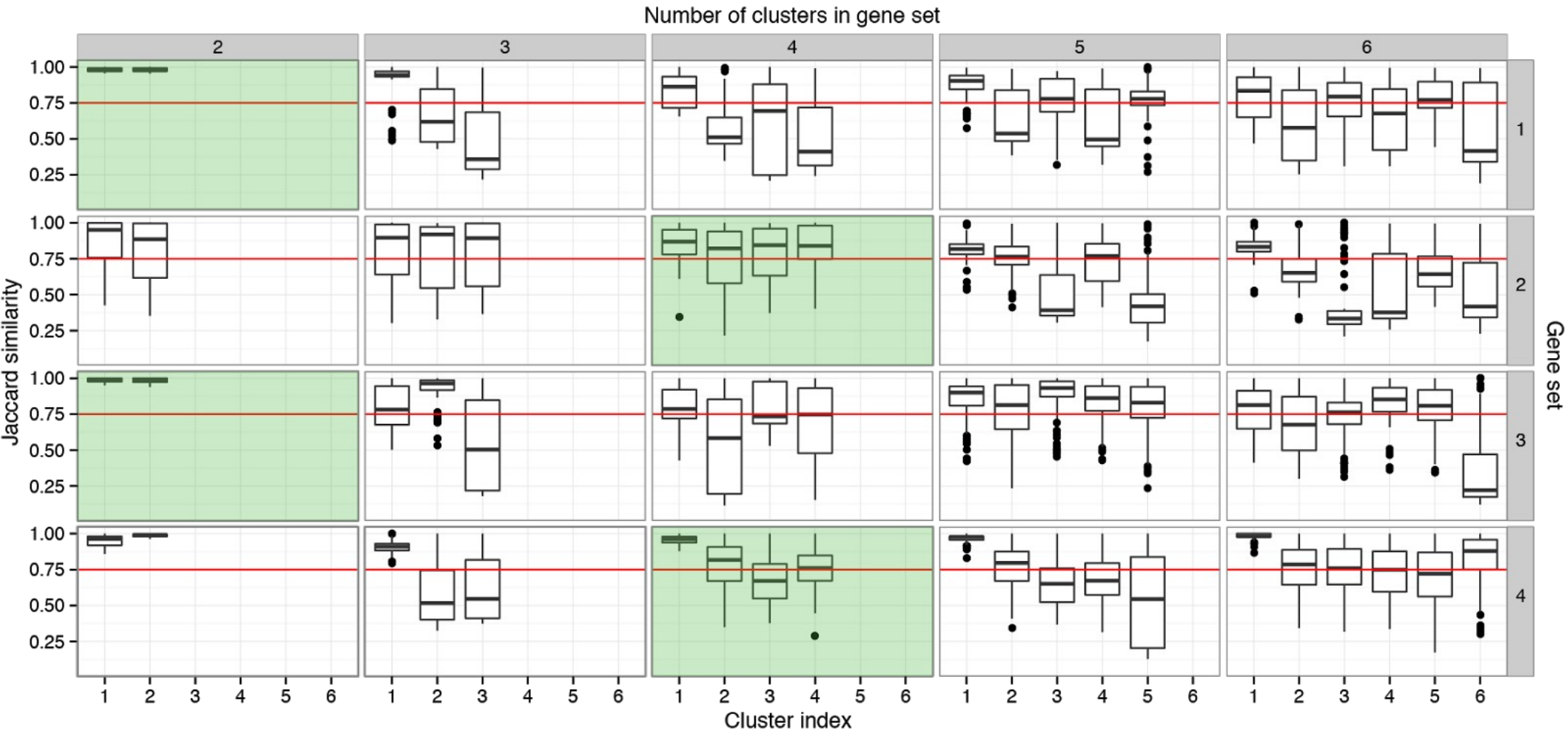
- Klijn *et al. Nat Biotechnol.* 2015
- ▲ Ours
- Hart *et al. PNAS* 2015

Condition

- H1047R
- PTEN-/-
- WT

All cells are isogenic MCF10a

Clustering A66



Number of clusters in gene set

