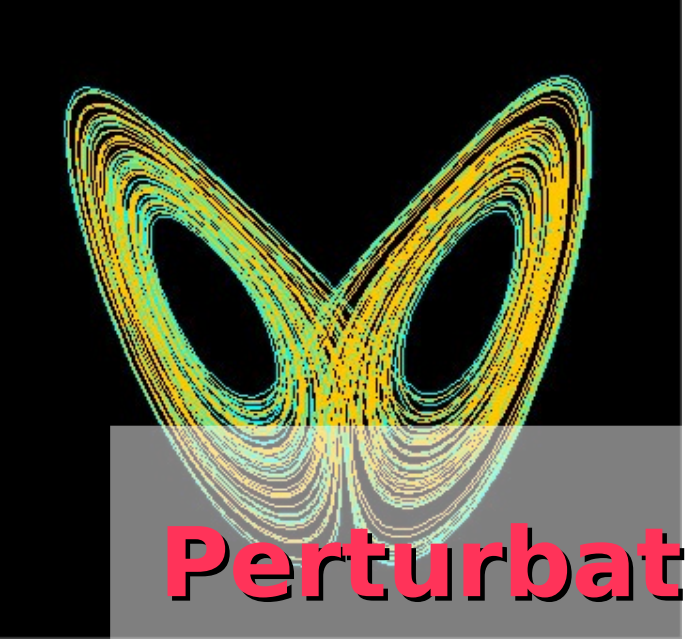
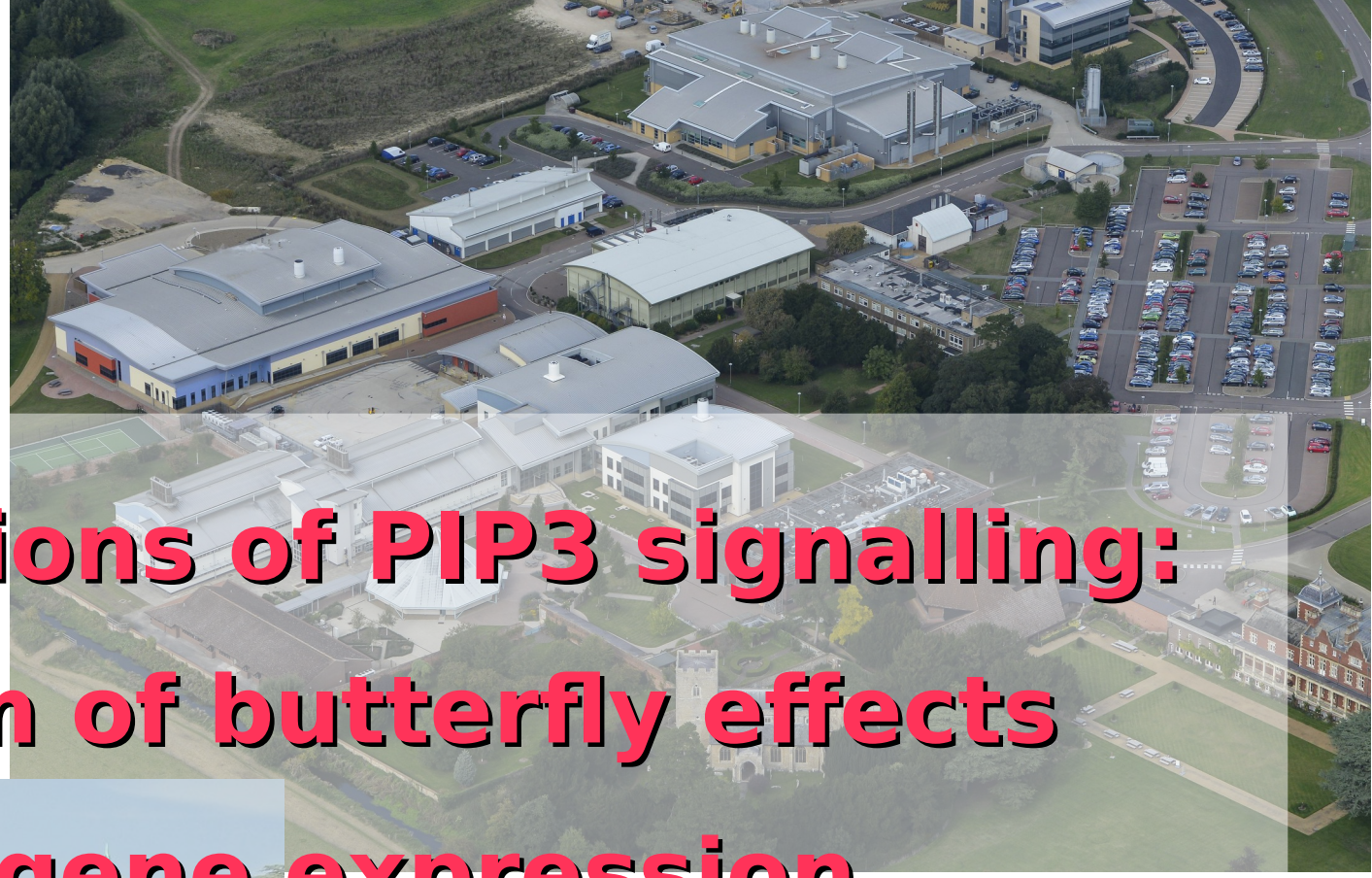
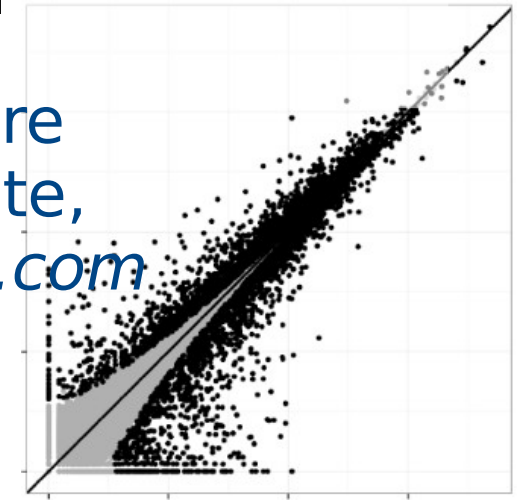




Perturbations of PIP3 signalling: a swarm of butterfly effects on gene expression

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



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eulife

BBSRC
bioscience for the future

Programmes in:

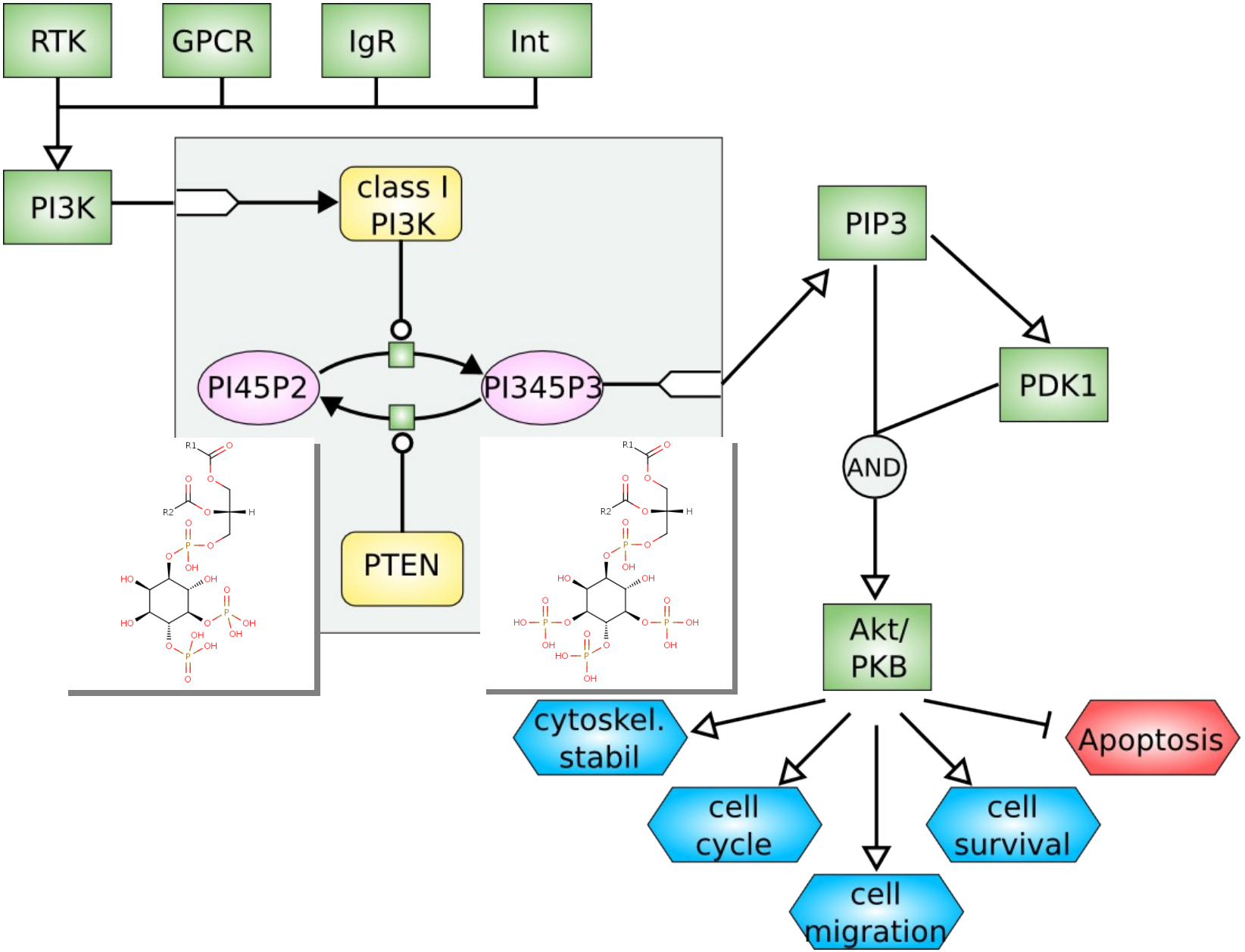
- **Signalling**
- **Immunology**
- **Epigenetics**
- **Nuclear Dynamics**

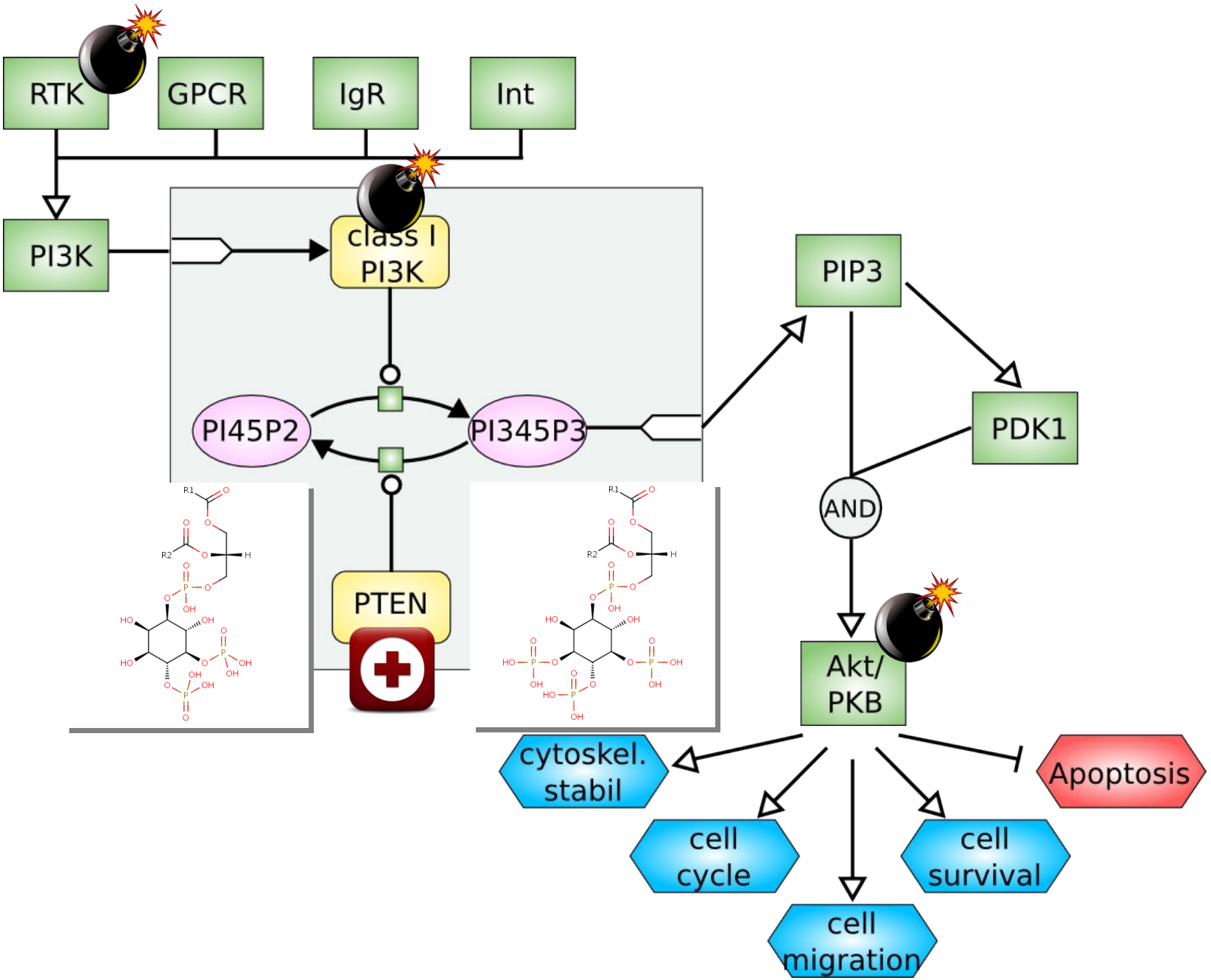
Plateformes including:

- **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



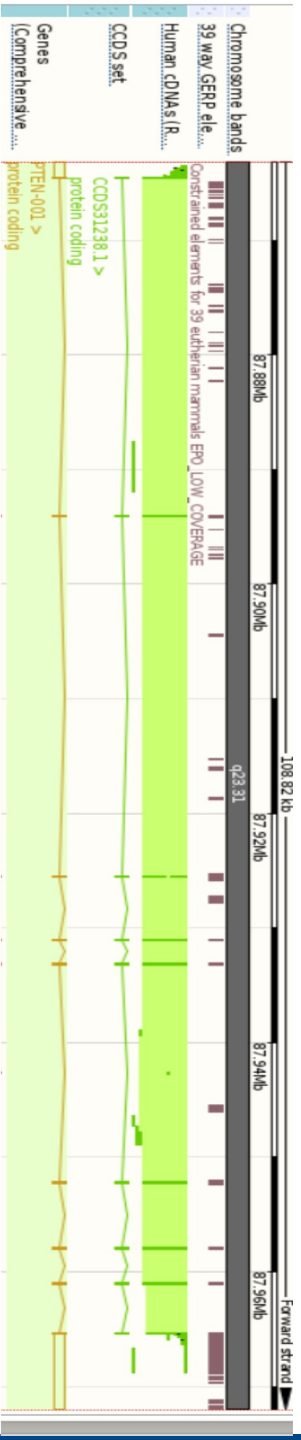

RTK
GPCR
IgR
Int
PI3K
class I PI3K
PIP3
PI45P2
PI345P3
PDK1
AND
PTEN
Akt/ PKB
cytoskel. stabil
Apoptosis
cell cycle
cell survival
cell migration

	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

- We identified a new phosphatase activity
- I am not going to talk about that at all

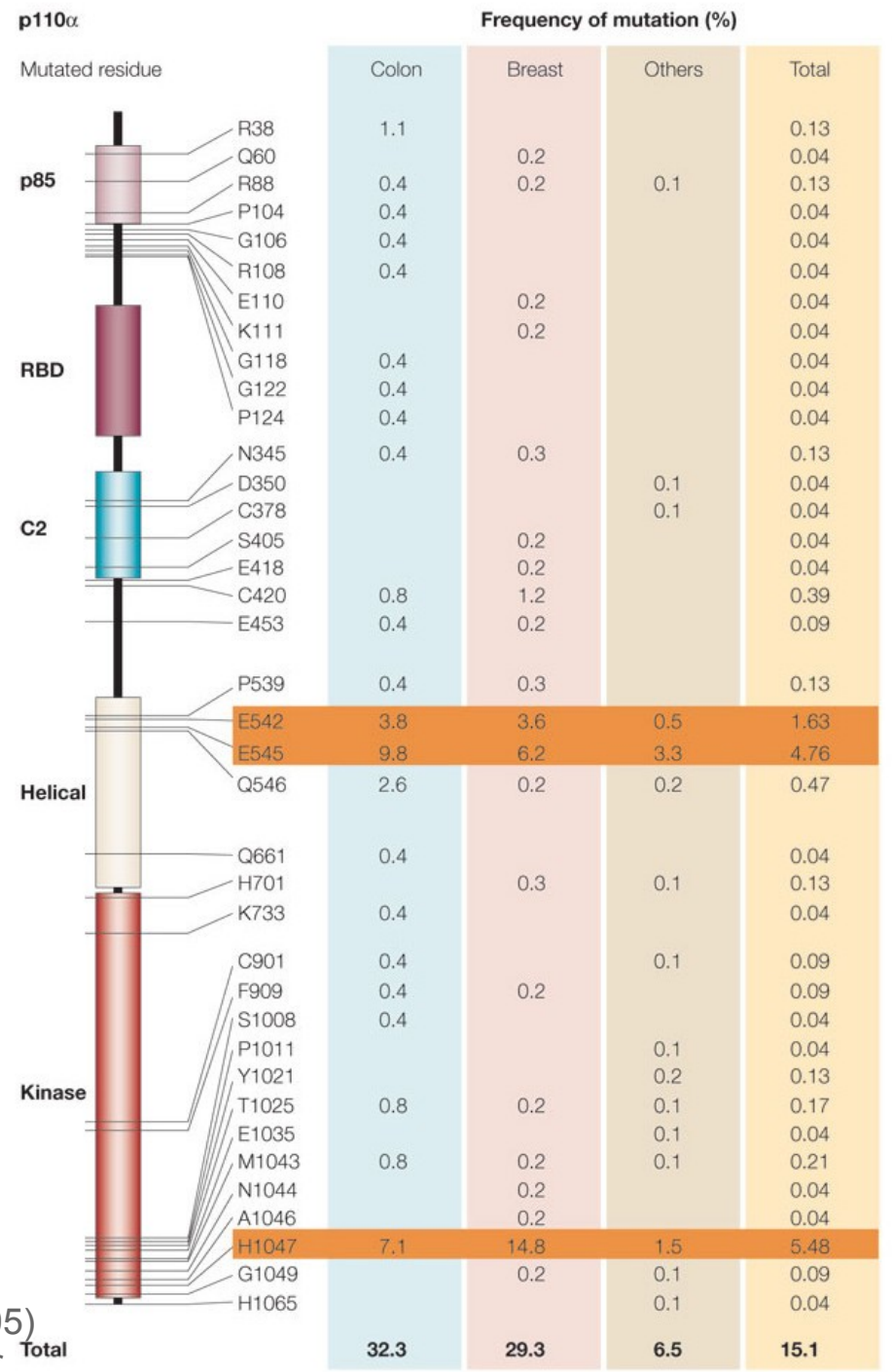
→ on to transcriptomics



PTEN

PI3K

Isogenic MCF10a cell lines

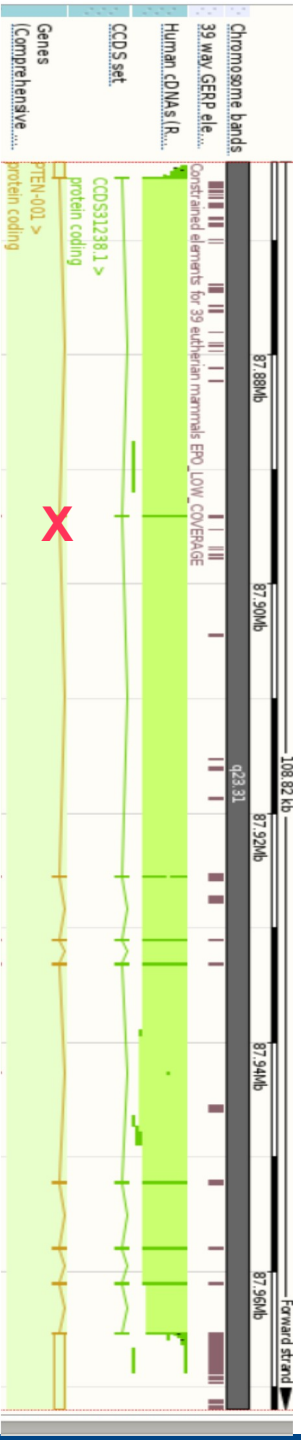


Bader *et al* (2005)
Nat Rev Cancer

Babraham Institute

PTEN

PI3K

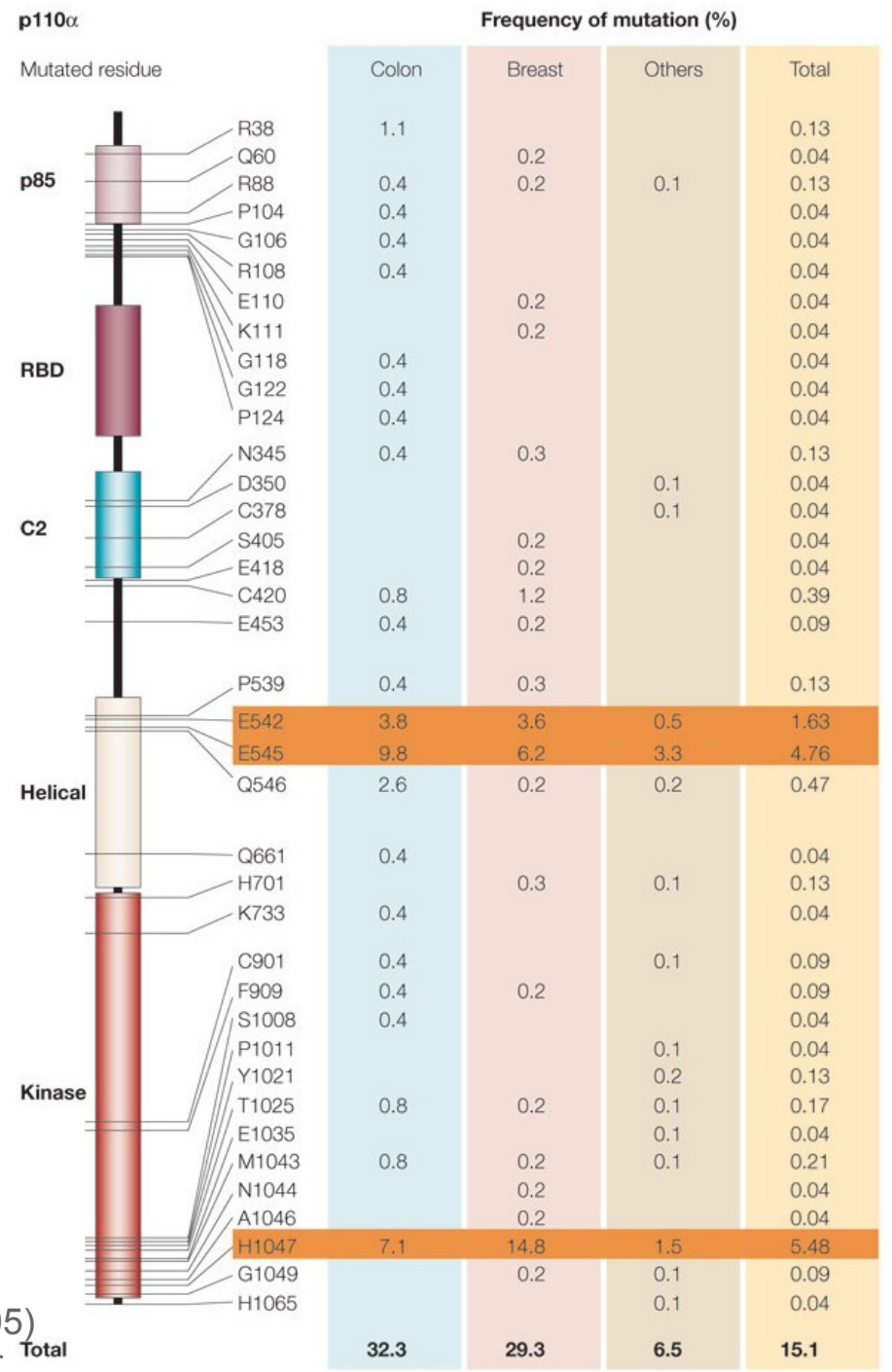


X

Isogenic MCF10a cell lines

phosphatase domain

inactivating bi-allelic mutation



Bader *et al* (2005)
Nat Rev Cancer

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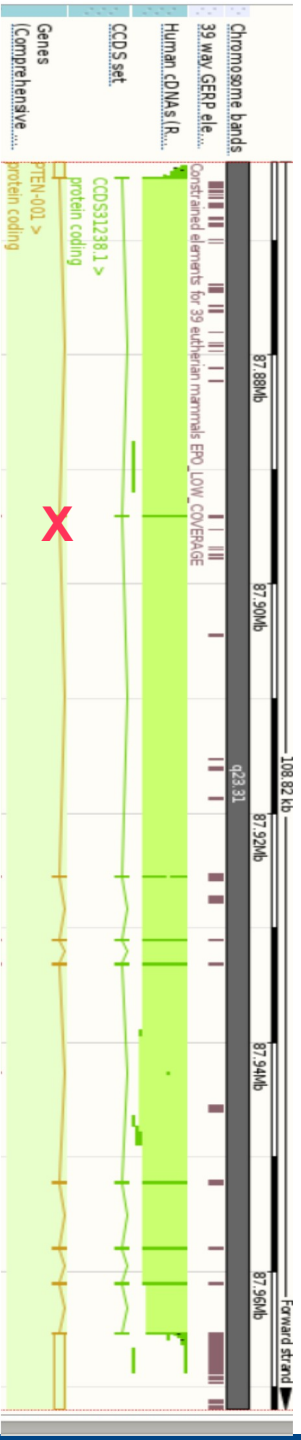
PTEN

PI3K

Isogenic MCF10a cell lines

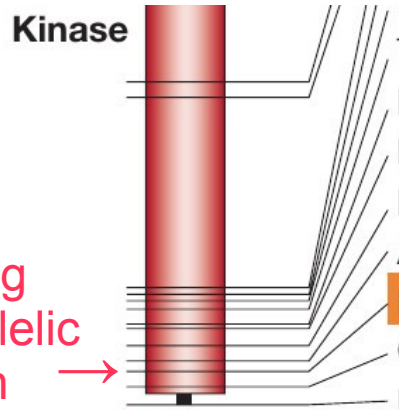


X

phosphatase domain

inactivating bi-allelic mutation

activating mono-allelic mutation →



p110α

Mutated residue		Frequency of mutation (%) Colon	Breast	Others	Total
p85	R38	1.1			0.13
	Q60		0.2		0.04
	R88	0.4	0.2	0.1	0.13
	P104	0.4			0.04
RBD	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
	P124	0.4			0.04
C2	N345	0.4	0.3		0.13
	D350			0.1	0.04
	C378			0.1	0.04
	S405		0.2		0.04
	E418		0.2		0.04
	C420	0.8	1.2		0.39
	E453	0.4	0.2		0.09
Helical	P539	0.4	0.3		0.13
	E542	3.8	3.6	0.5	1.63
	E545	9.8	6.2	3.3	4.76
	Q546	2.6	0.2	0.2	0.47
	Q661	0.4			0.04
	H701		0.3	0.1	0.13
	K733	0.4			0.04
	C901	0.4		0.1	0.09
Kinase	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

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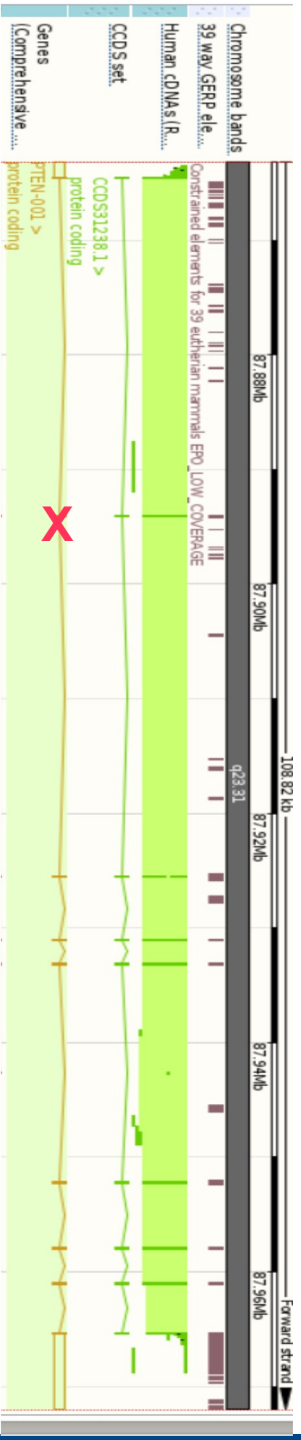
PTEN

PI3K

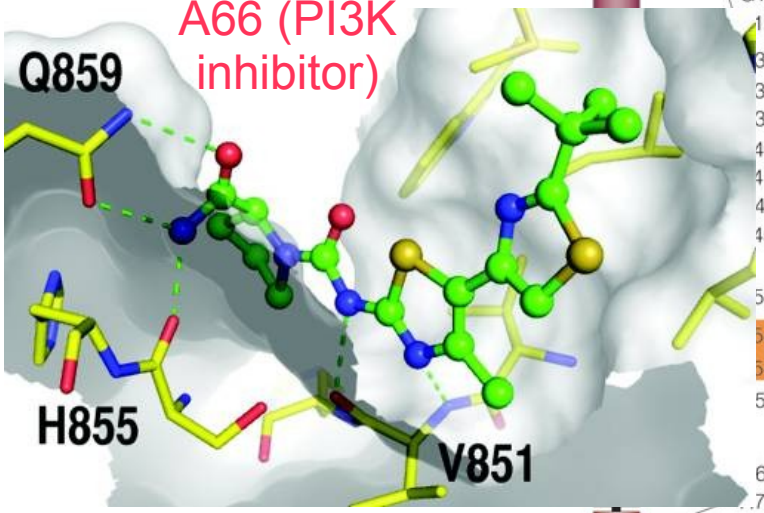
Isogenic MCF10a cell lines

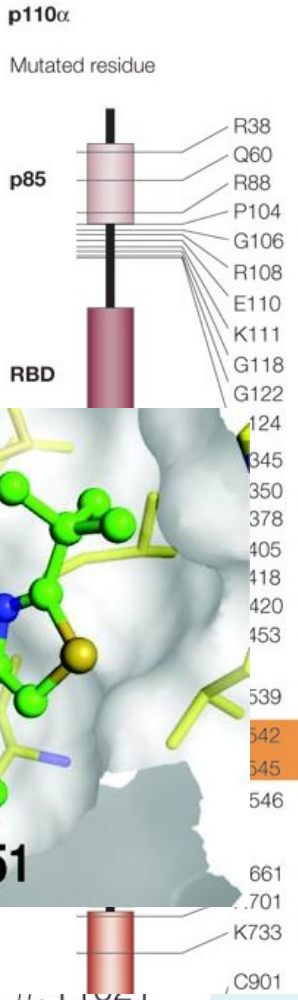


X

phosphatase domain

inactivating bi-allelic mutation





Frequency of mutation (%)				
Mutated residue	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

activating mono-allelic mutation →

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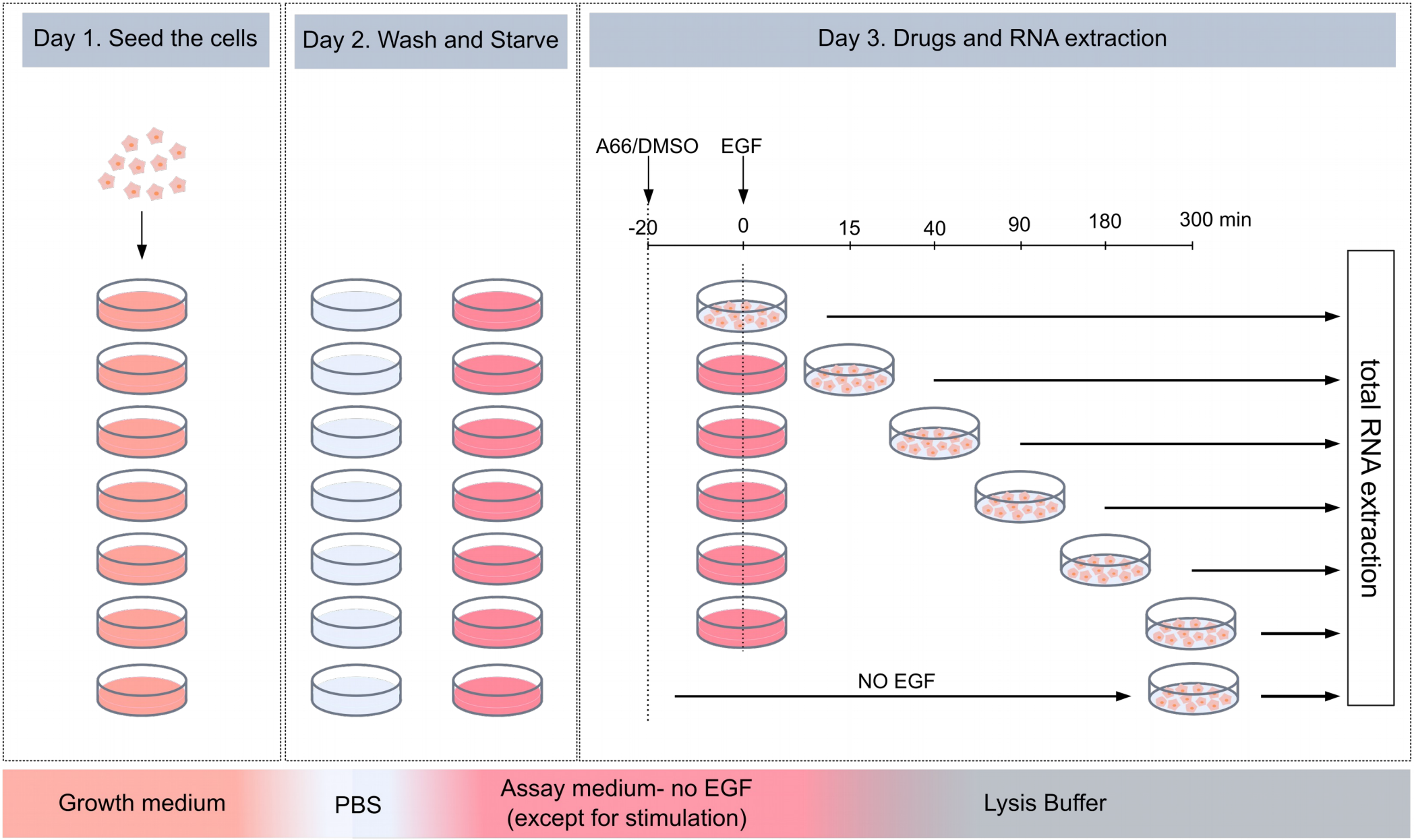
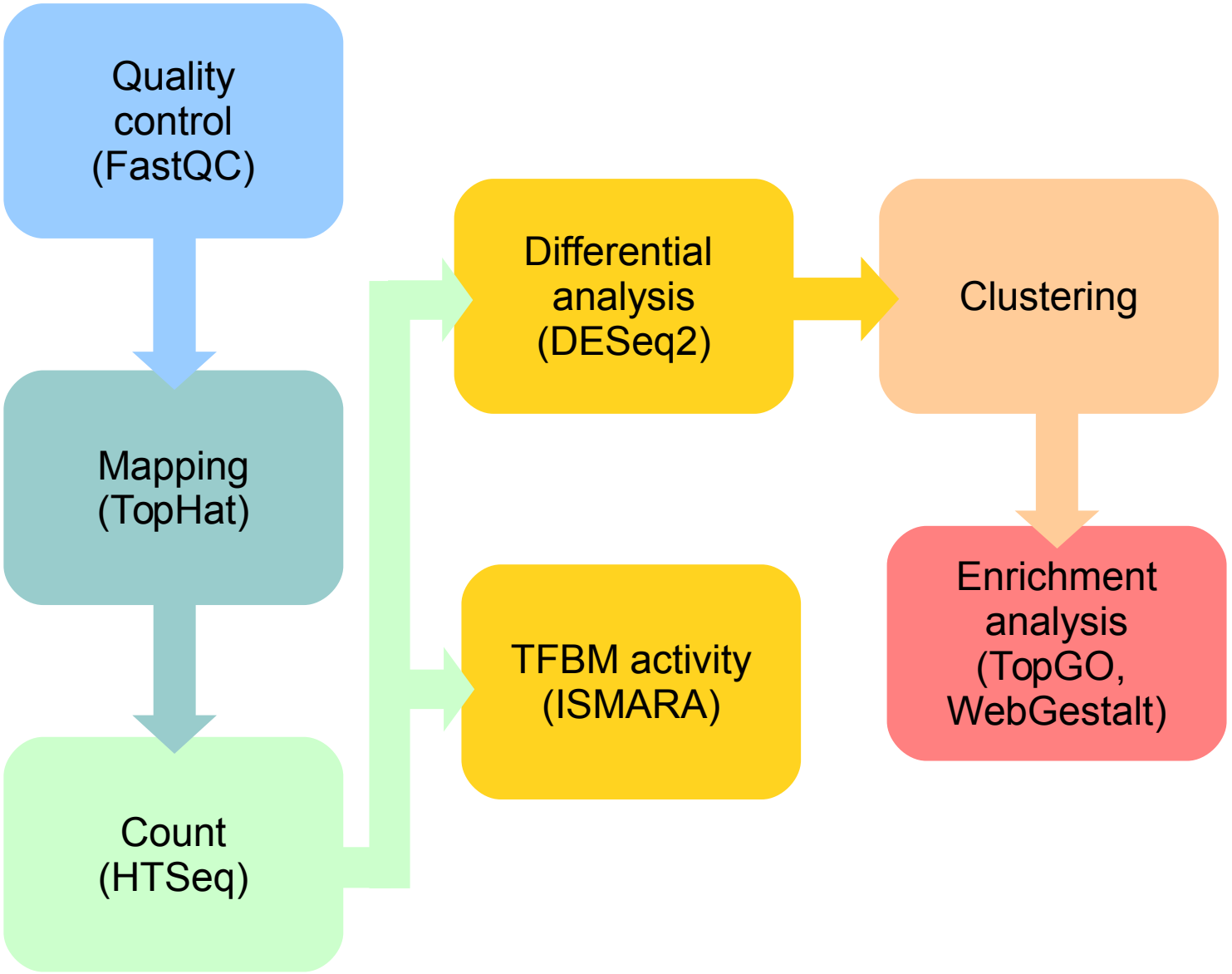
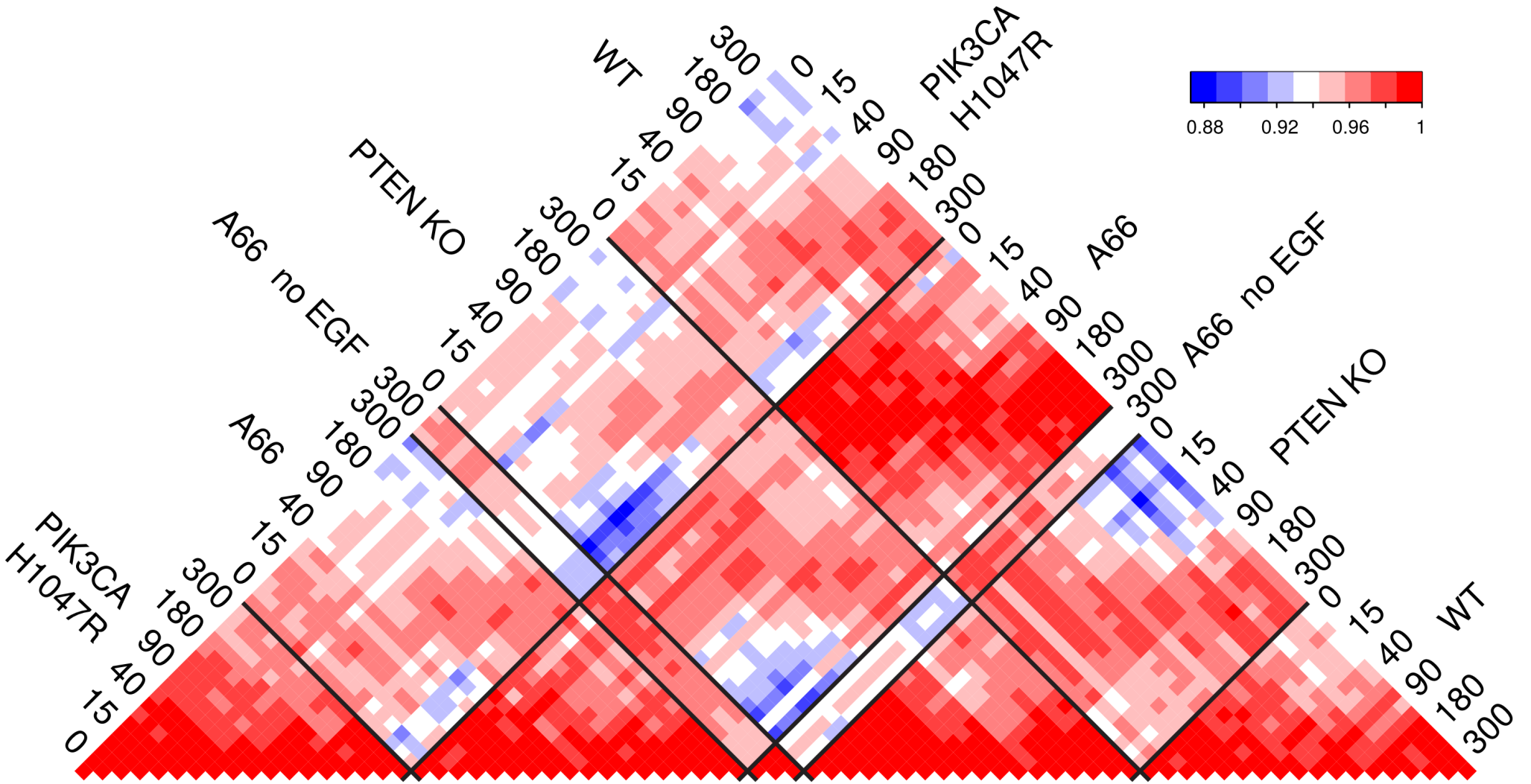
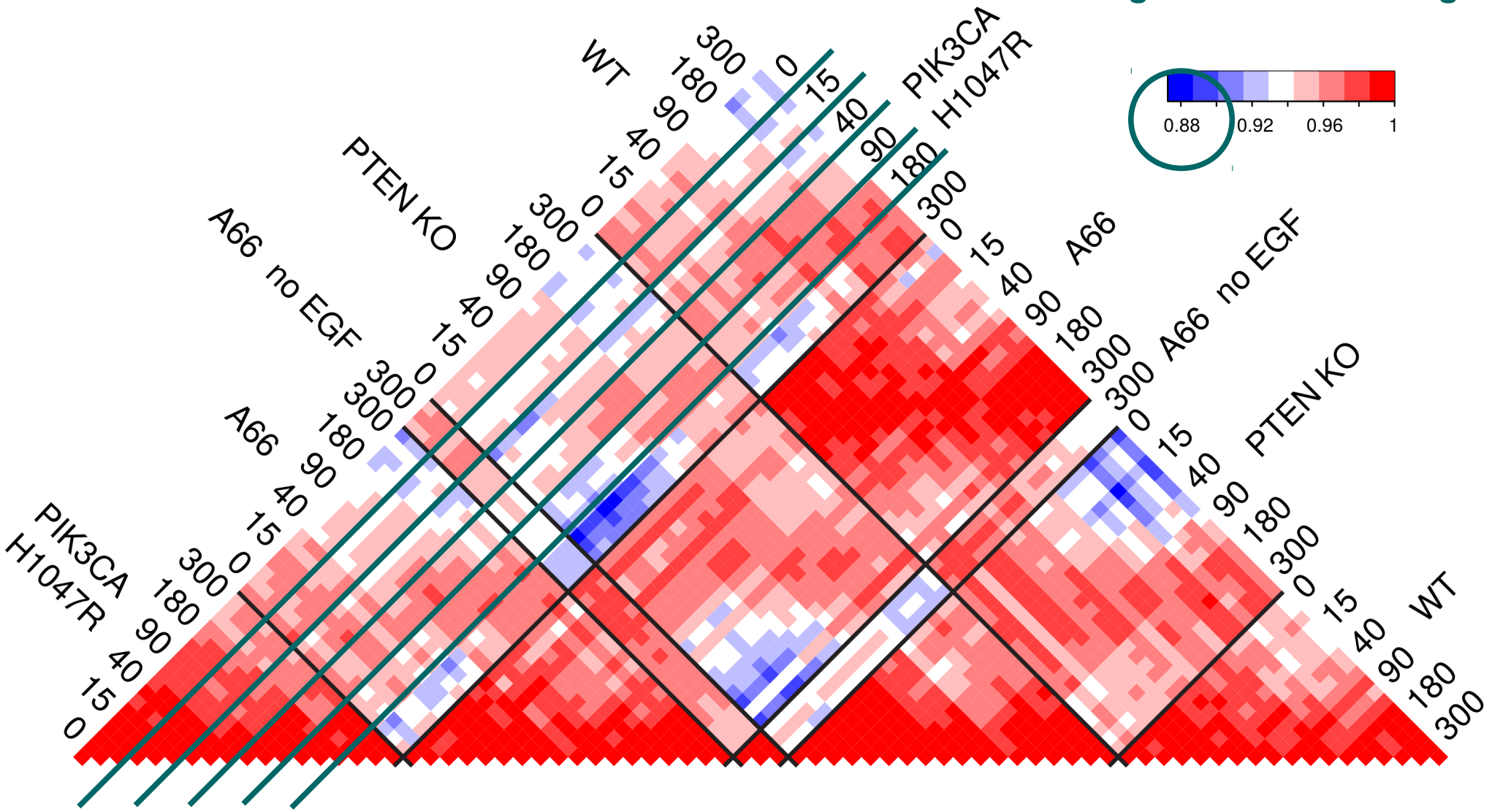

Day 1. Seed the cells
Day 2. Wash and Starve
Day 3. Drugs and RNA extraction
A66/DMSO
EGF
-20
0
15
40
90
180
300 min
NO EGF
total RNA extraction
Growth medium
PBS
Assay medium- no EGF
(except for stimulation)
Lysis Buffer


Quality control (FastQC)
Mapping (TopHat)
Count (HTSeq)
Differential analysis (DESeq2)
TFBM activity (ISMARA)
Clustering
Enrichment analysis (TopGO, WebGestalt)



Most genes do not change



replicates are OK

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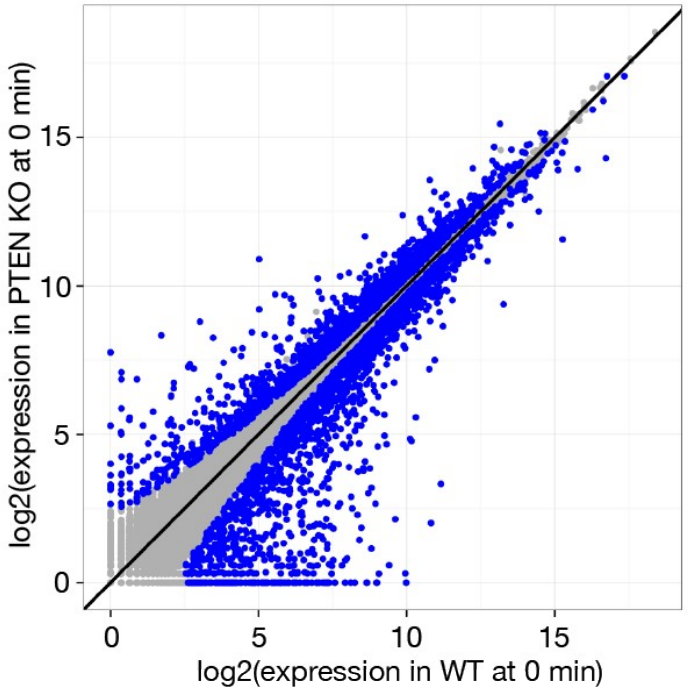
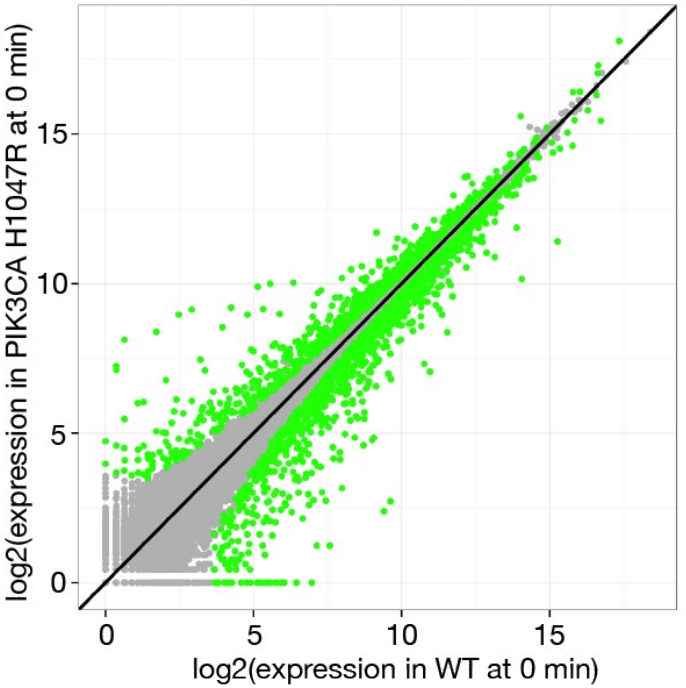
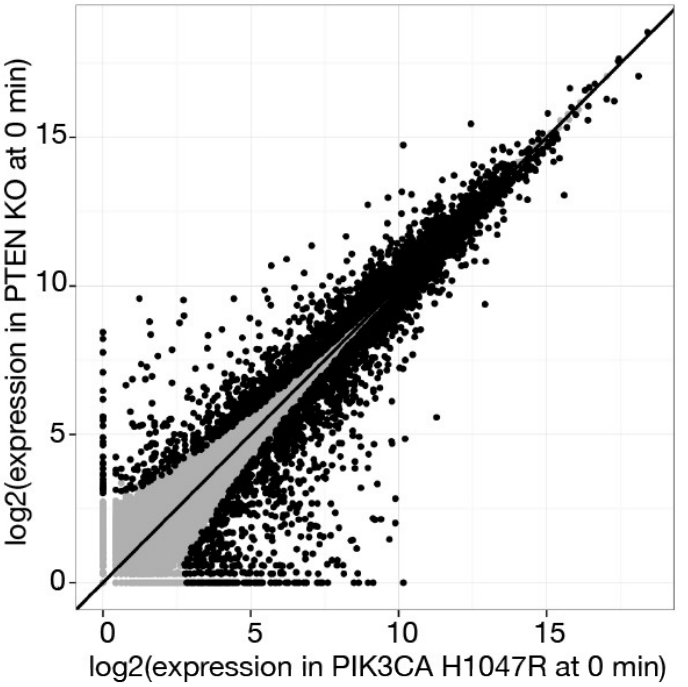
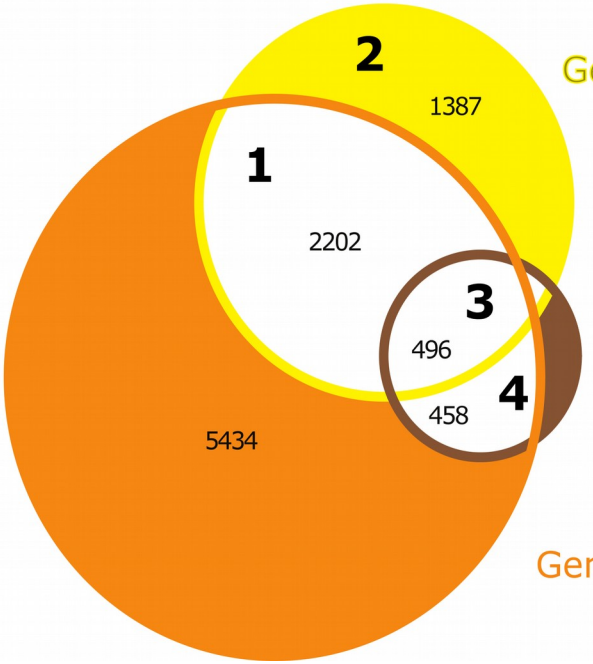
But quite a few are affected nevertheless









Genes affected by PI3K basal activity.

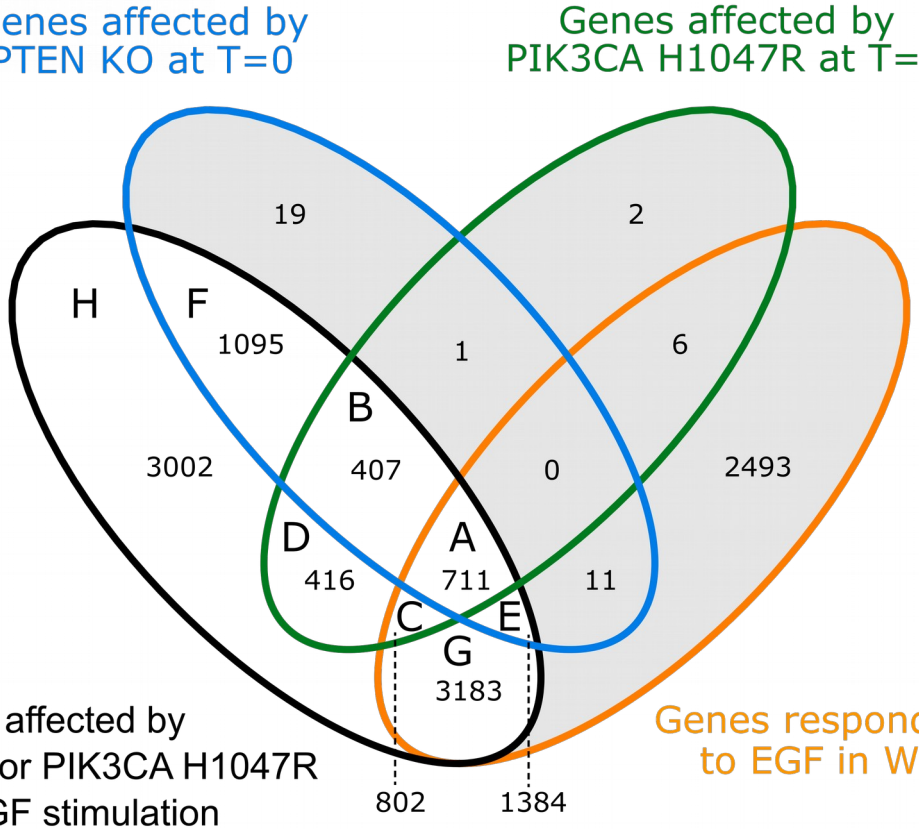
Genes affected by EGF-induced activity.

Genes responding to EGF in WT.

4725 genes affected by A66

1543 genes affected by H1047R

2244 genes affected by PTEN-/-



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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 [a]Molecular and Experimental Medicine and [b]Chemical 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 p110α 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

http://www.bioinformatics.babraham.ac.uk/shiny/kiselev-pip3-rna-seq-gene-profiles/

Gene expression profiles in MCF10A cells upon EGF stimulation

Gene HGNC symbol / Ensembl ID

LBH

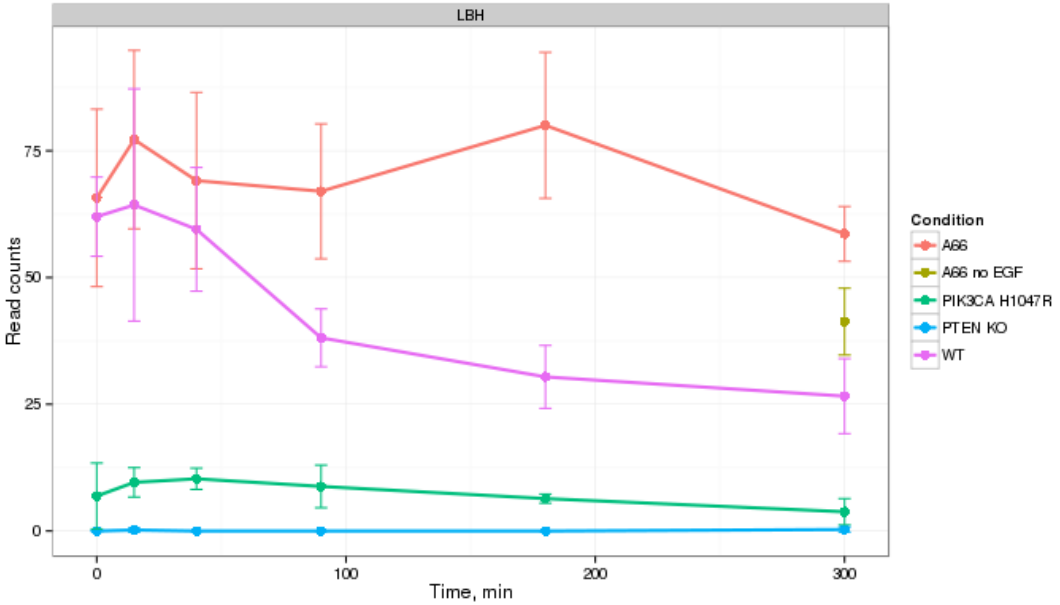
RPKM

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)



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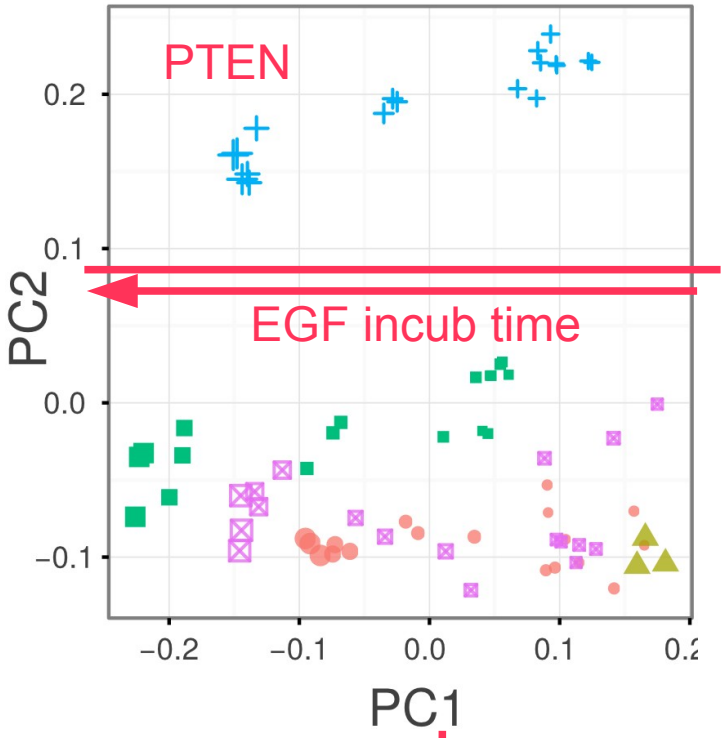
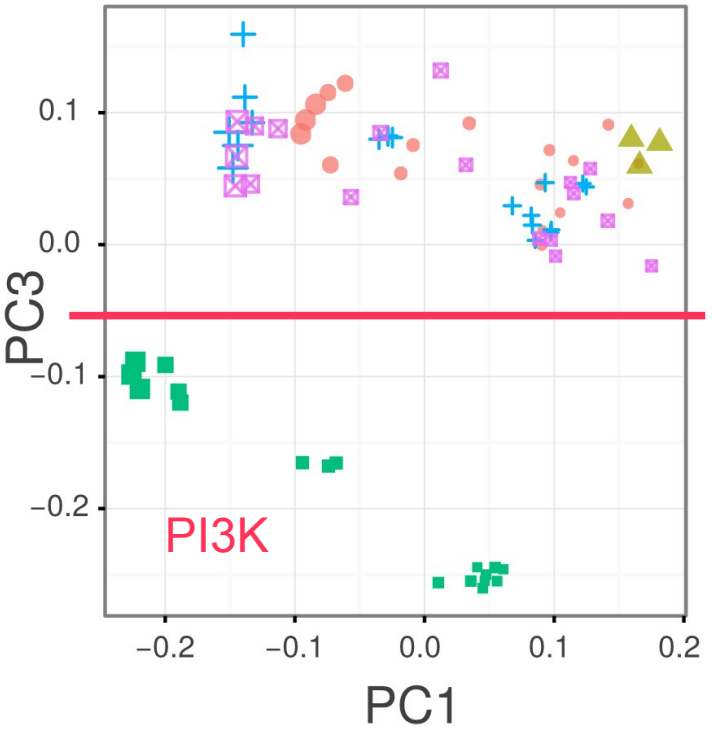
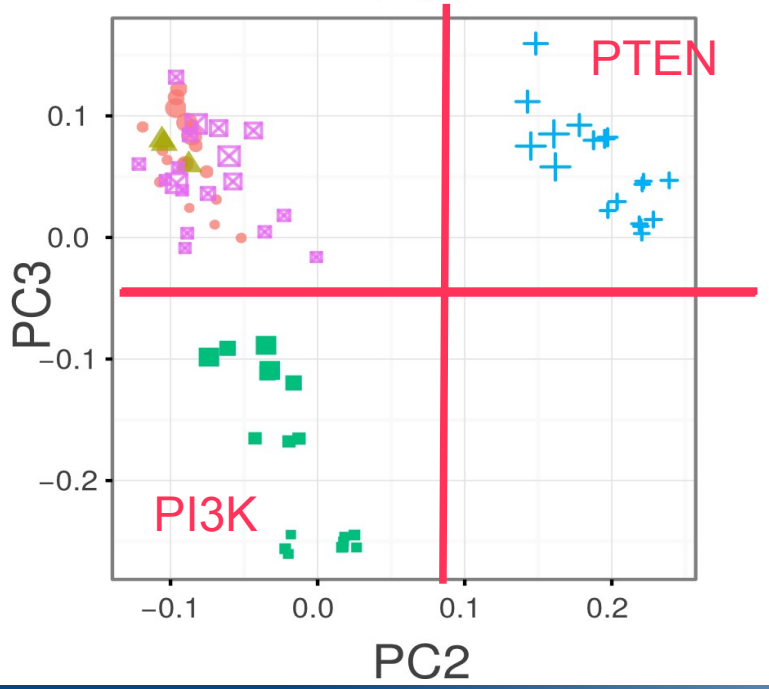


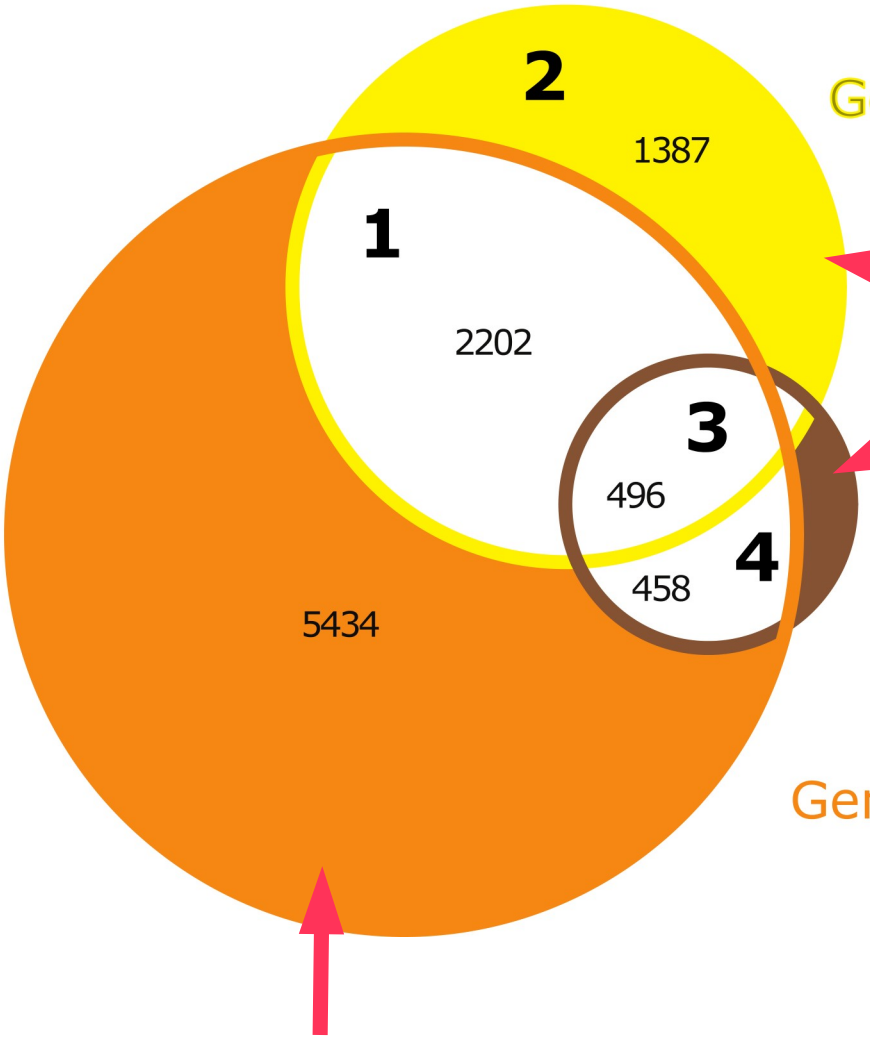




Time, min
- 0
- 100
- 200
- 300

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

Effect of acute PI3K inhibition



Genes affected by PI3K basal activity.

A66 effect

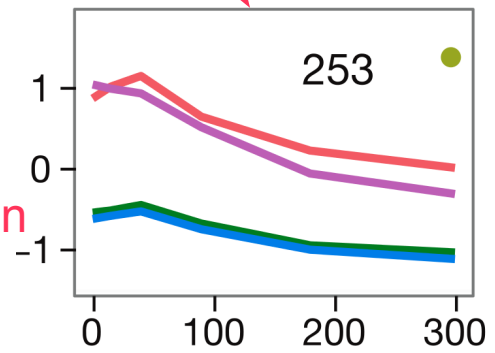
Genes affected by EGF-induced activity.

Genes responding to EGF in WT.

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

What am I showing you on the next slides?

one cluster,
comprising 253 genes



centred
normal
expression

time of EGF treatment

WT

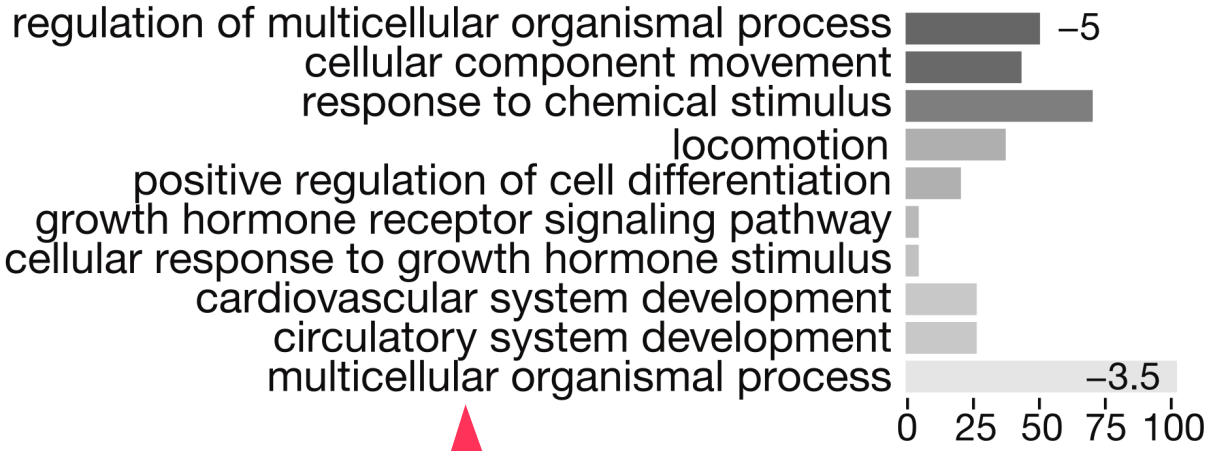
WT+A66

PI3K H1045R

WT+A66

A66 no EGF

log p-value
(darker = more significant)



Gene Ontology
Biological Process

number of genes
with this GO term

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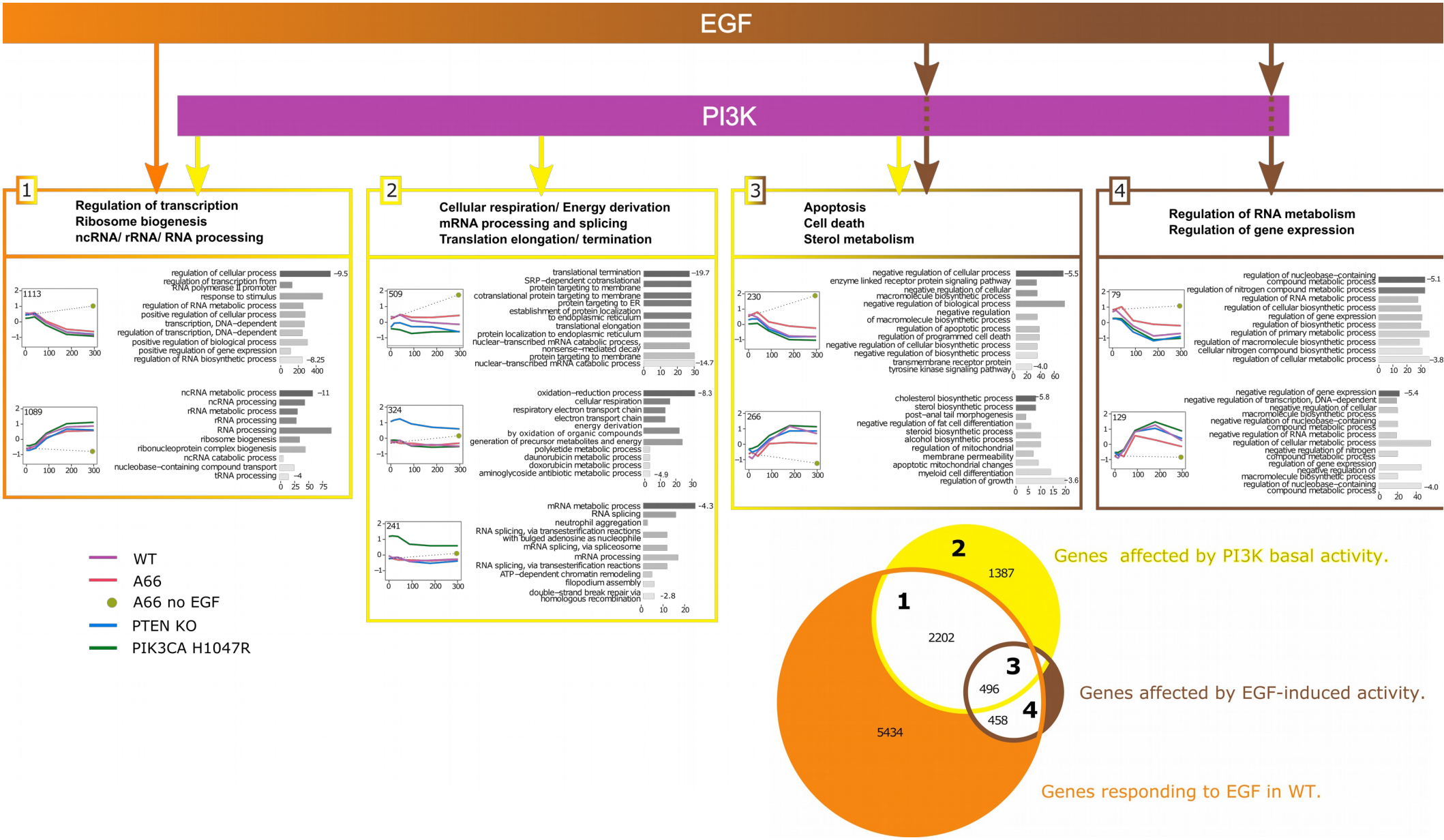

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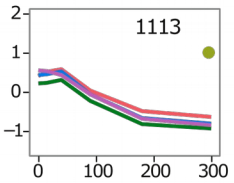
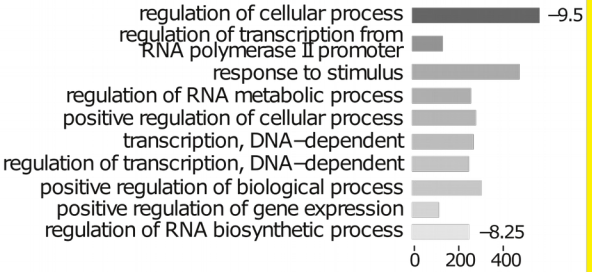
EC

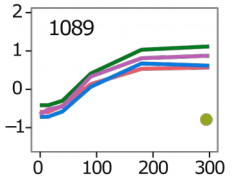
PI

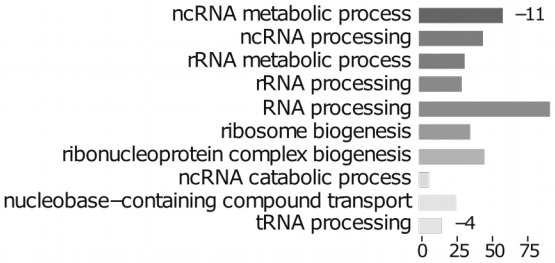
1

Regulation of transcription
Ribosome biogenesis
ncRNA/ rRNA/ RNA processing



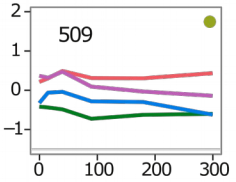


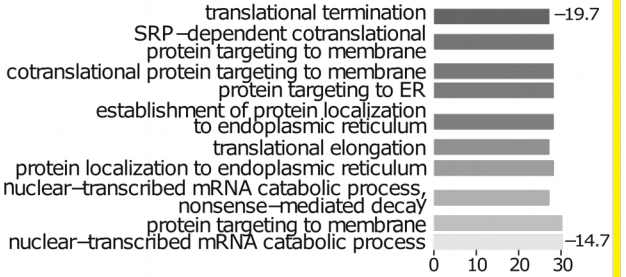


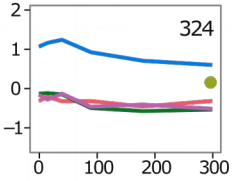


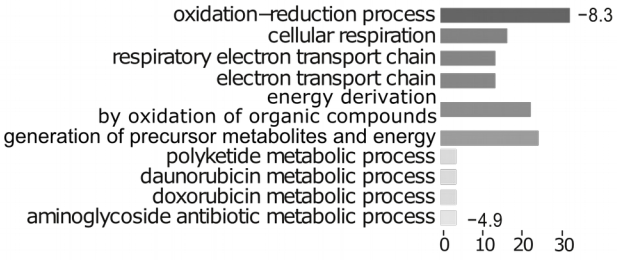
2

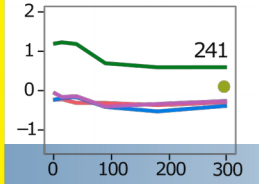
Cellular respiration/ Energy derivation
mRNA processing and splicing
Translation elongation/ termination

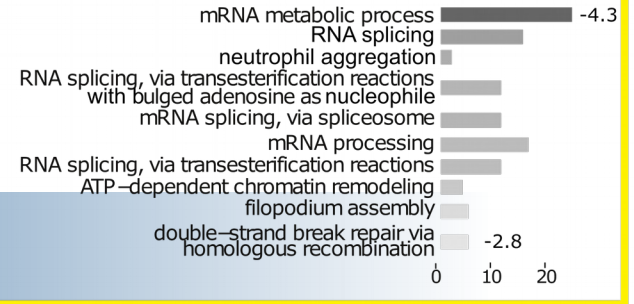












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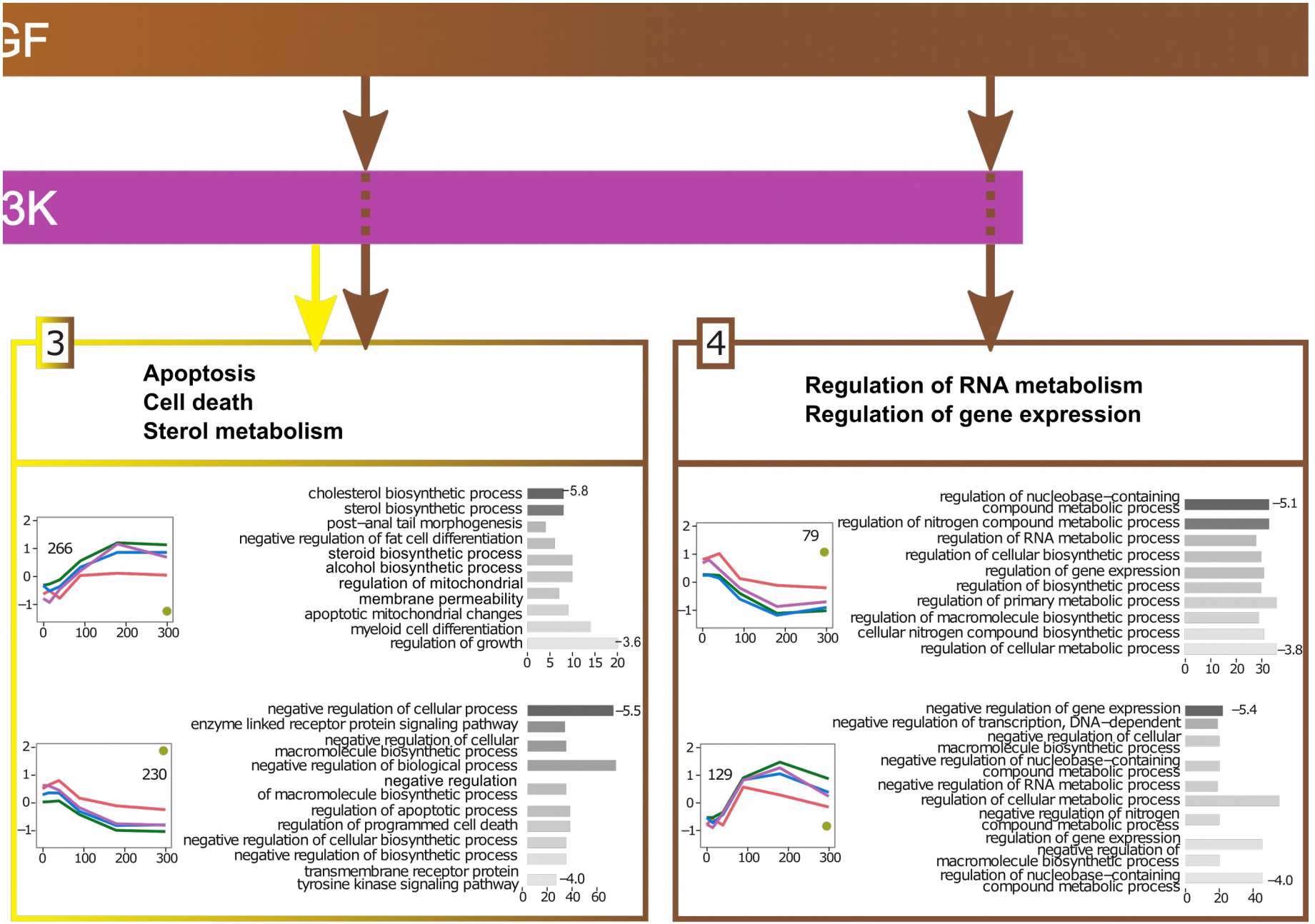
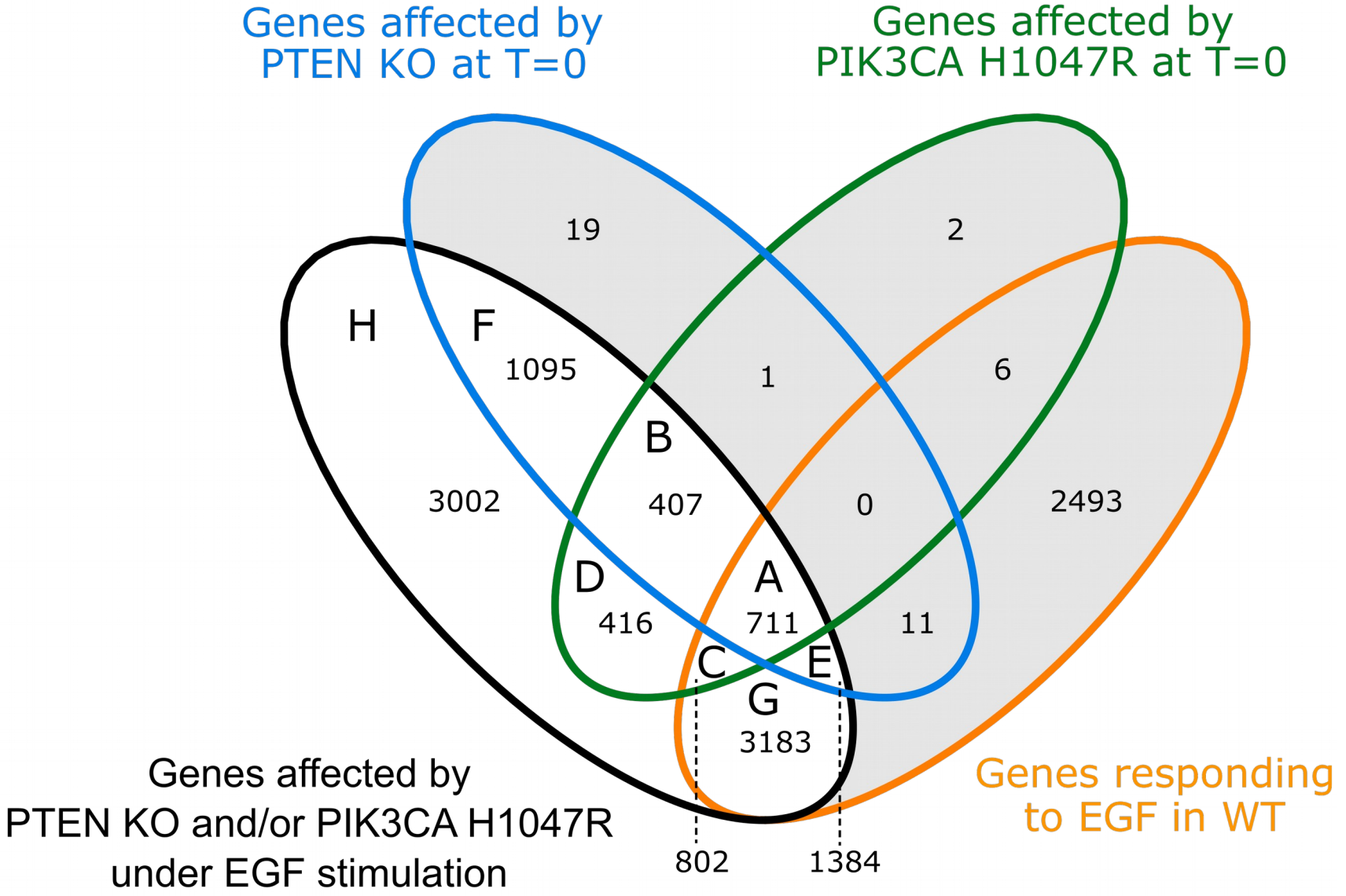

GF
3K
3
Apoptosis
Cell death
Sterol metabolism
266
cholesterol biosynthetic process
sterol biosynthetic process
post–anal tail morphogenesis
negative regulation of fat cell differentiation
steroid biosynthetic process
alcohol biosynthetic process
regulation of mitochondrial
membrane permeability
apoptotic mitochondrial changes
myeloid cell differentiation
regulation of growth
5.8
3.6
230
negative regulation of cellular process
enzyme linked receptor protein signaling pathway
negative regulation of cellular
macromolecule biosynthetic process
negative regulation of biological process
negative regulation
of macromolecule biosynthetic process
regulation of apoptotic process
regulation of programmed cell death
negative regulation of cellular biosynthetic process
negative regulation of biosynthetic process
transmembrane receptor protein
tyrosine kinase signaling pathway
5.5
4.0
4
Regulation of RNA metabolism
Regulation of gene expression
79
regulation of nucleobase–containing
compound metabolic process
regulation of nitrogen compound metabolic process
regulation of RNA metabolic process
regulation of cellular biosynthetic process
regulation of gene expression
regulation of biosynthetic process
regulation of primary metabolic process
regulation of macromolecule biosynthetic process
cellular nitrogen compound biosynthetic process
regulation of cellular metabolic process
5.1
3.8
129
negative regulation of gene expression
negative regulation of transcription, DNA–dependent
negative regulation of cellular
macromolecule biosynthetic process
negative regulation of nucleobase–containing
compound metabolic process
negative regulation of RNA metabolic process
regulation of cellular metabolic process
negative regulation of nitrogen
compound metabolic process
regulation of gene expression
negative regulation of
macromolecule biosynthetic process
regulation of nucleobase–containing
compound metabolic process
5.4
4.0

Effects of constitutive mutations



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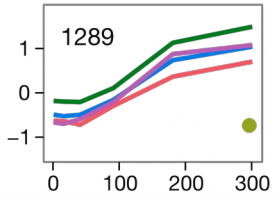
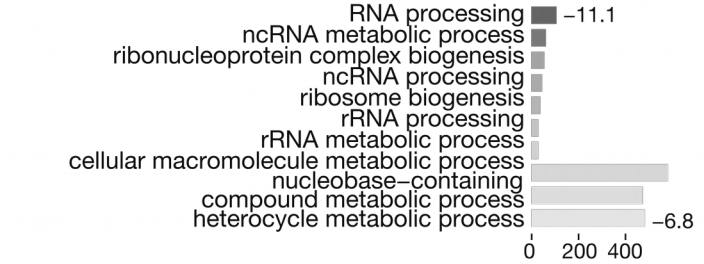
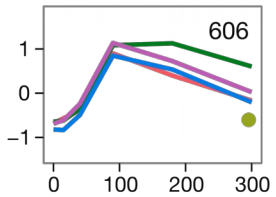
PIK3CA H1047R + PTEN KO

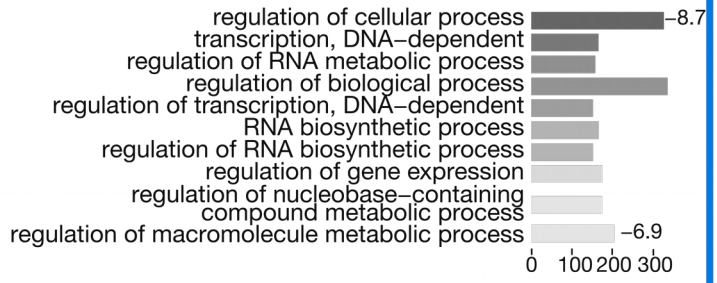
G

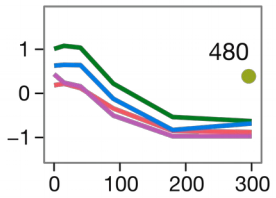
ncRNA/ rRNA/ RNA processing
Ribosome biogenesis
Transcription

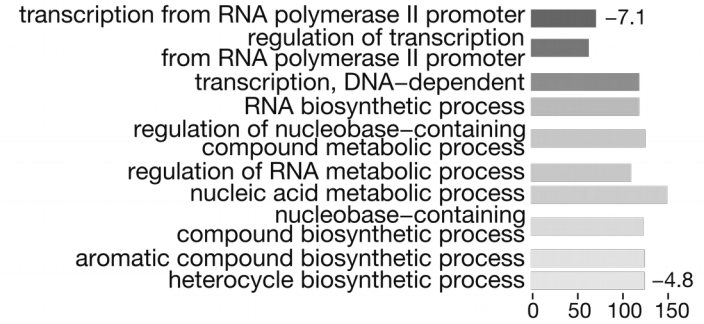






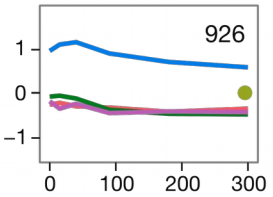


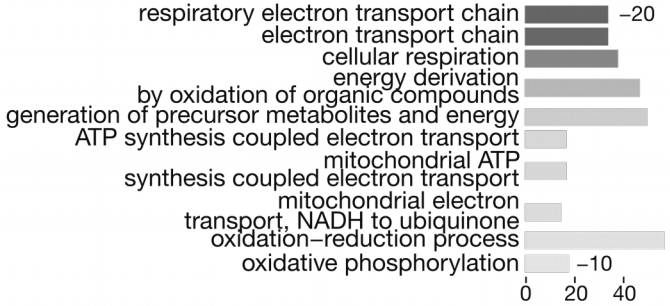


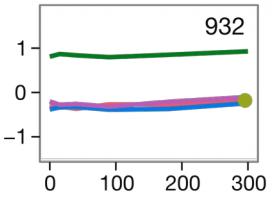


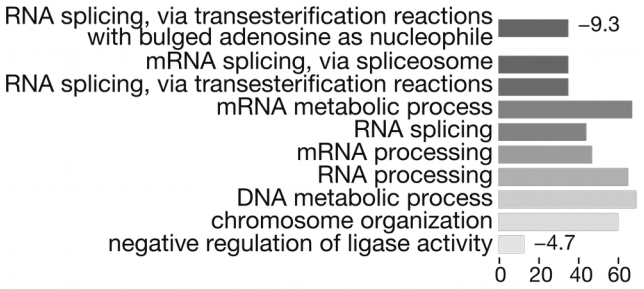
H

Cell respiration
Energy derivation
mRNA splicing/ processing









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

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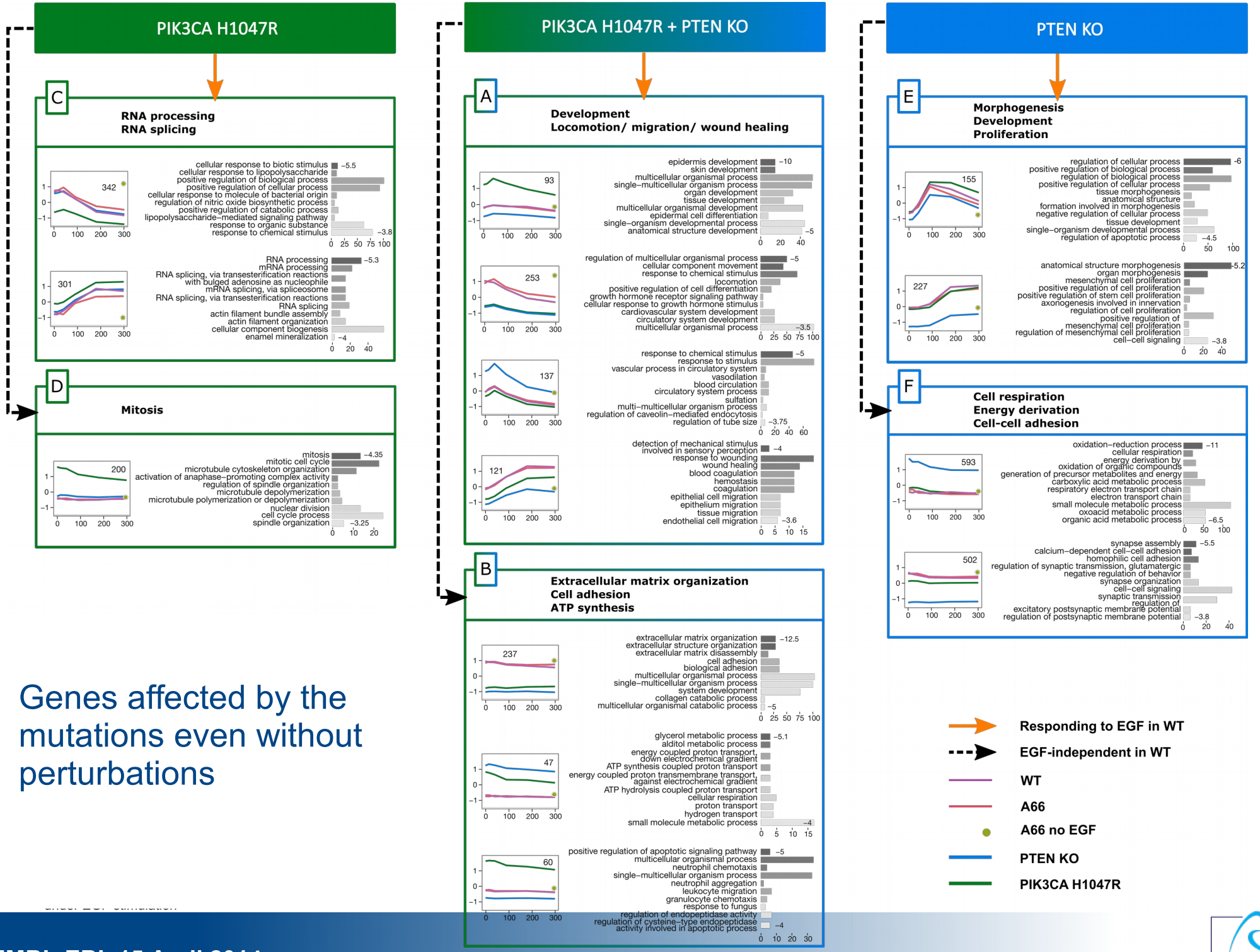
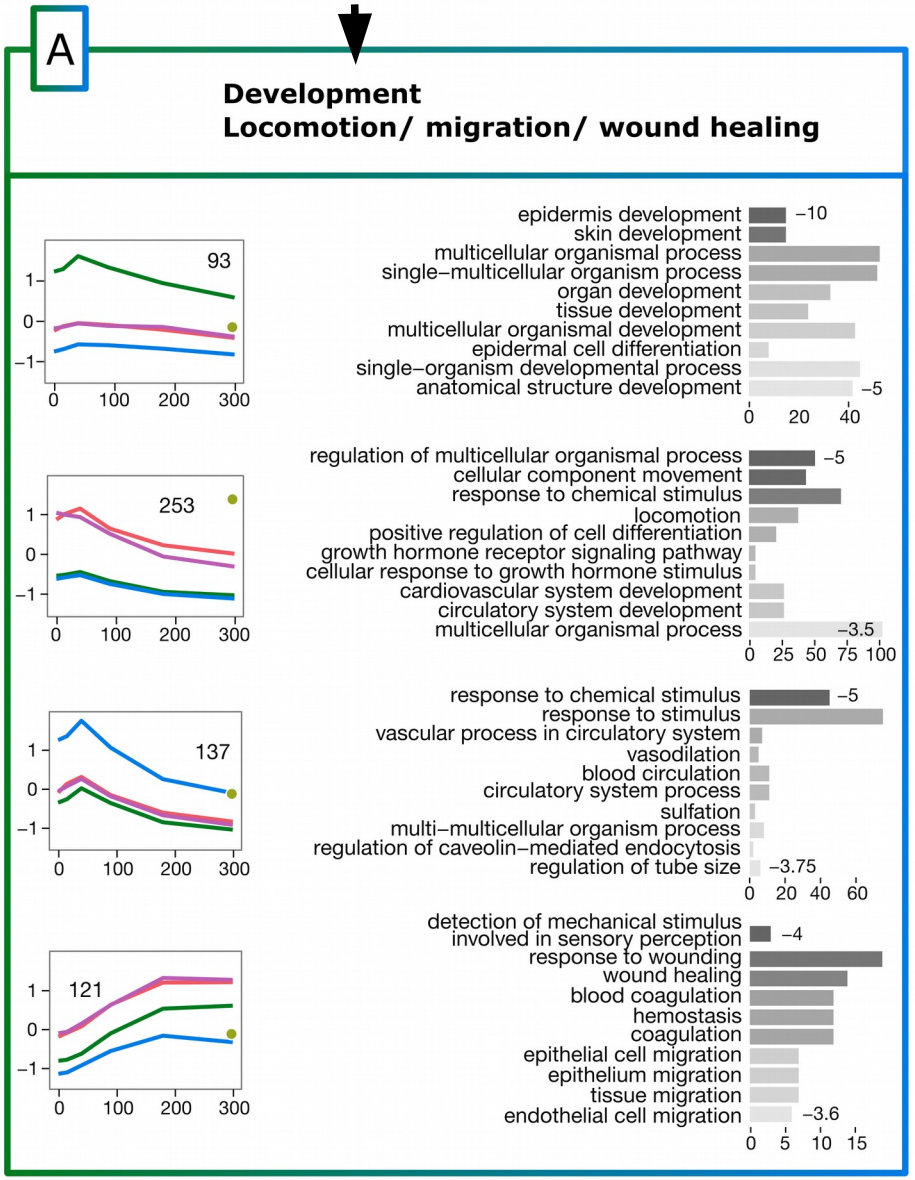
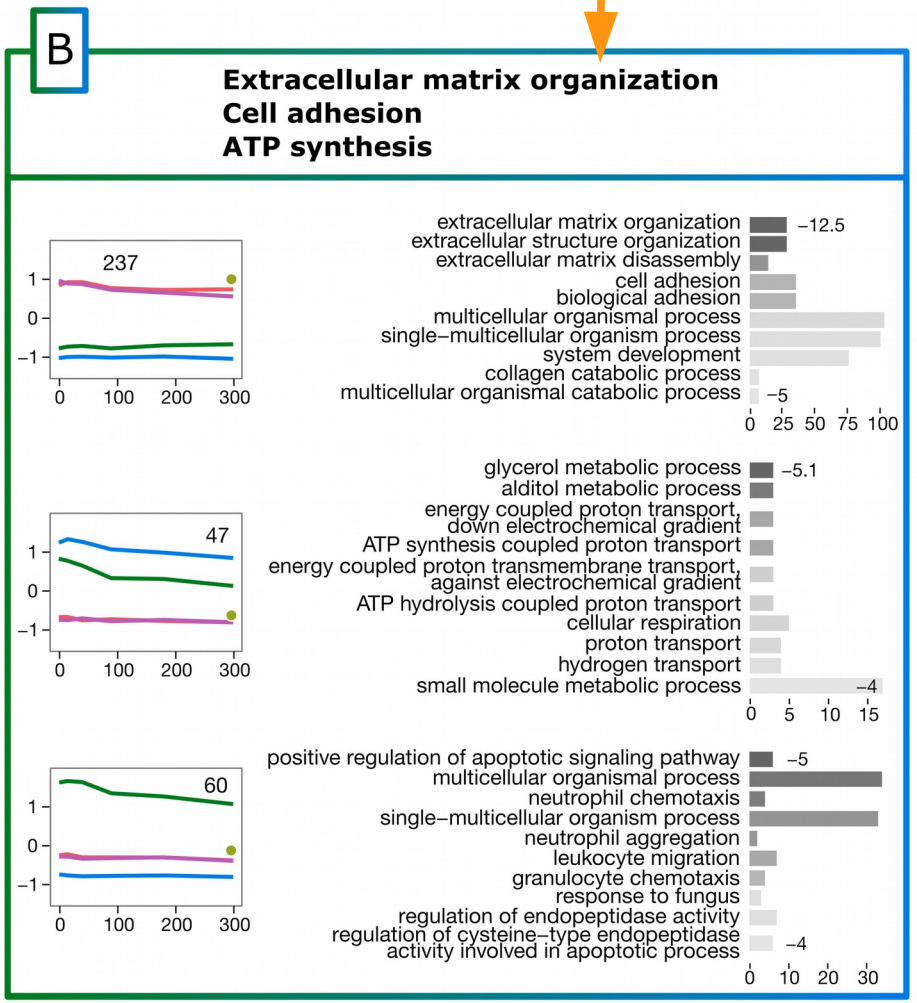


Genes affected by the mutations even without perturbations

PIK3CA H1047R + PTEN KO





Genes responding to both mutations in a coherent fashion

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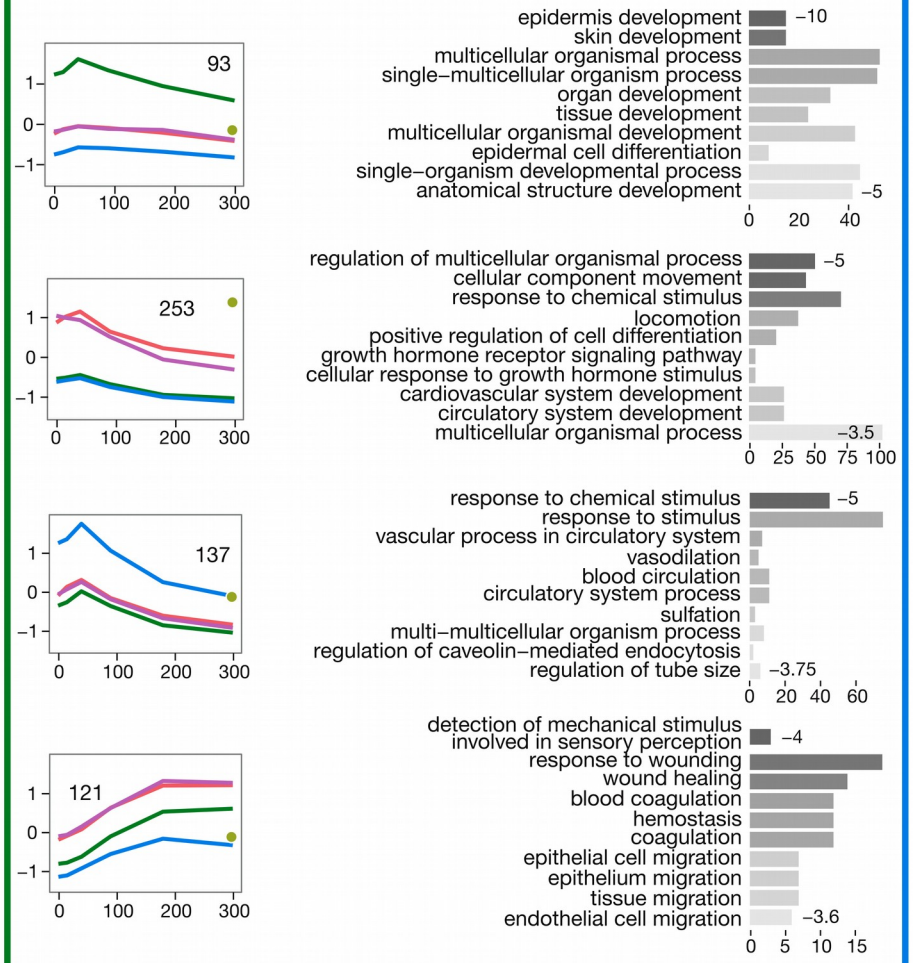
PIK3CA H1047R + PTEN KO

DYNAMIC

A

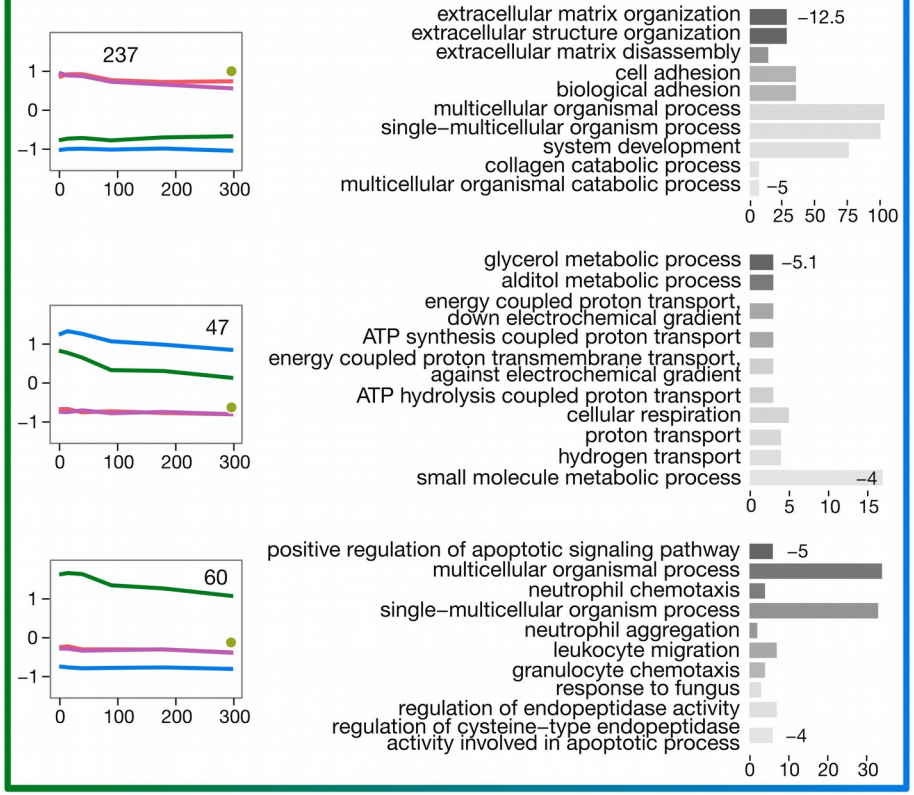
Development
Locomotion/ migration/ wound healing



STATIC

B

Extracellular matrix organization
Cell adhesion
ATP synthesis



Genes responding to both mutations in a coherent fashion

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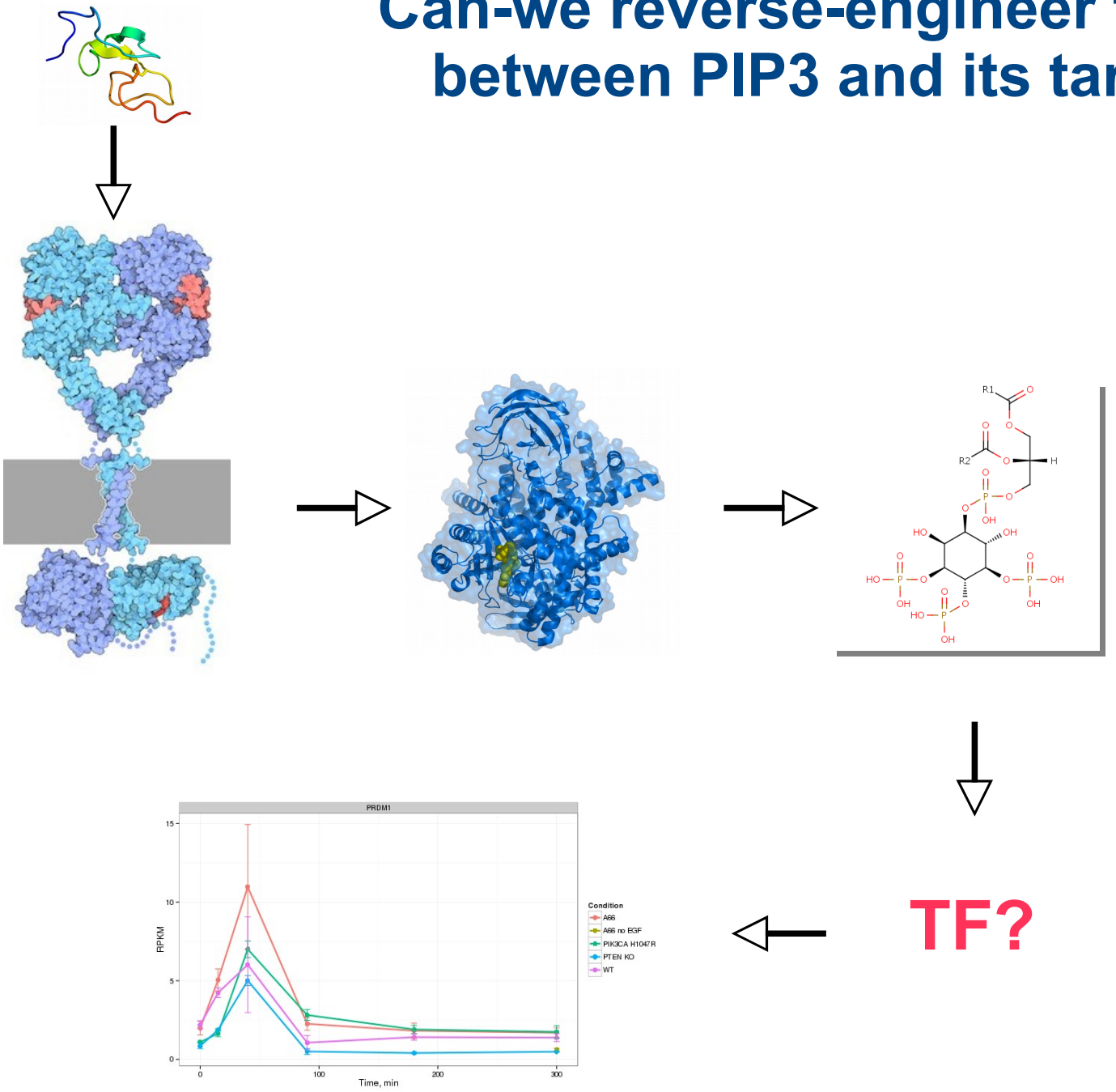
Can-we reverse-engineer the link between PIP3 and its targets?



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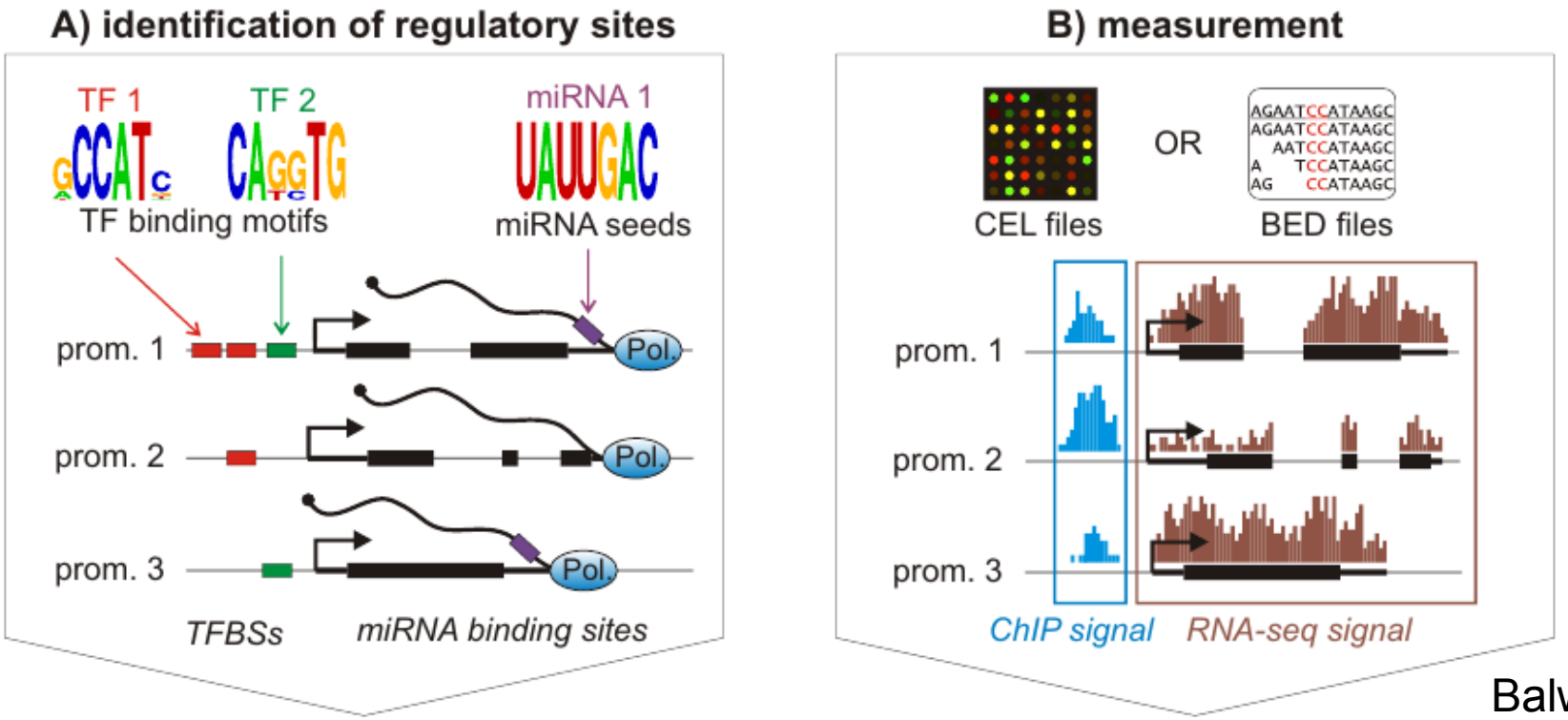
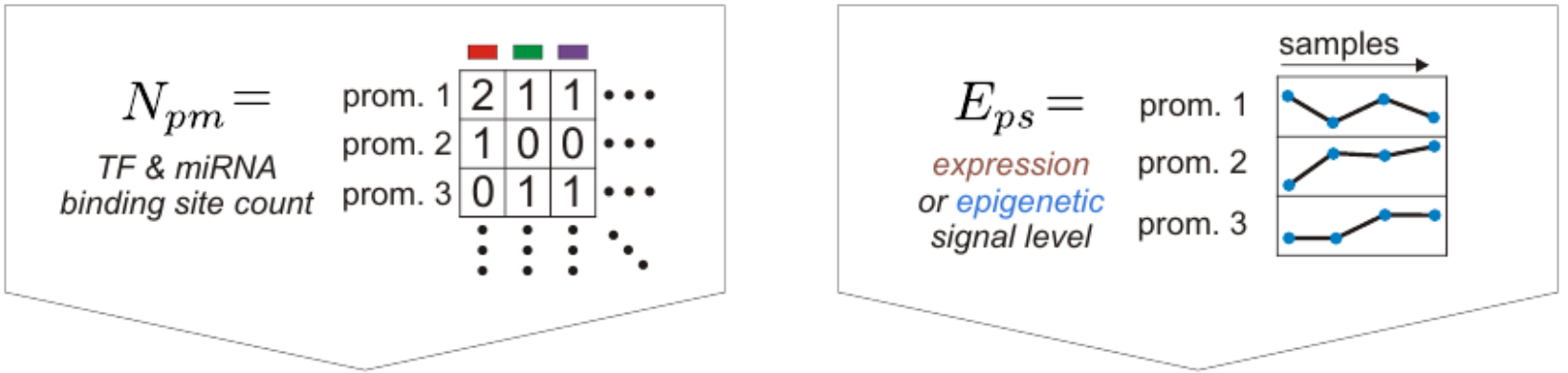


Balwiertz *et al* (2014) Genome Res



D) MARA model

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

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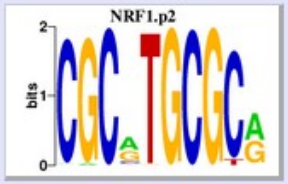
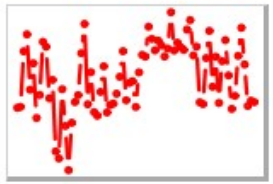
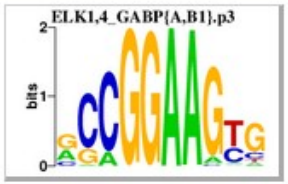
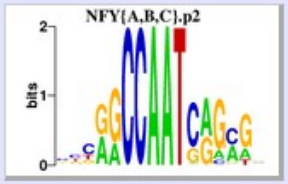
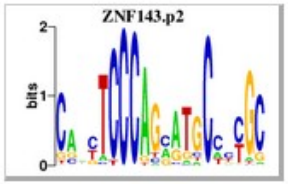
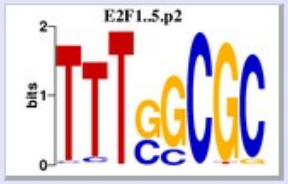
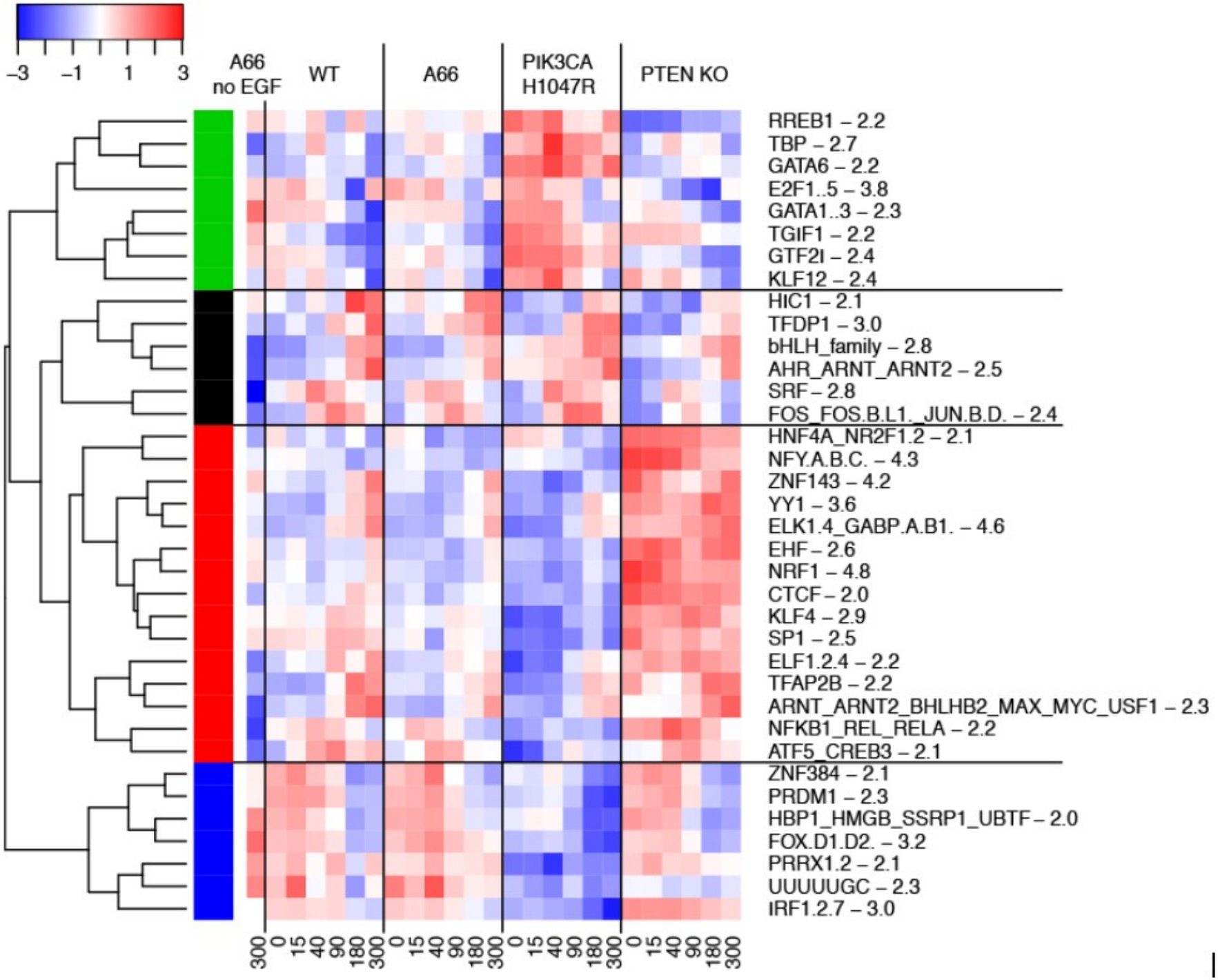
Motif name	Z-value	Associated genes	Profile	Logo
NRF1.p2	4.755	NRF1 (EWG, ALPHA-PAL)		
ELK1,4_GABP{A,B1}.p3	4.633	GABPA (E4TF1A, NFT2, NRF2, E4TF1-60, NRF2A) GABPB1 (E4TF1-47, GABPB) ELK4 (SAP1) ELK1		
NFY{A,B,C}.p2	4.292	NFYC (CBF-C) NFYB (CBF-A, HAP3) NFYA (HAP2, CBF-B)		
ZNF143.p2	4.154	ZNF143 (SBF,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



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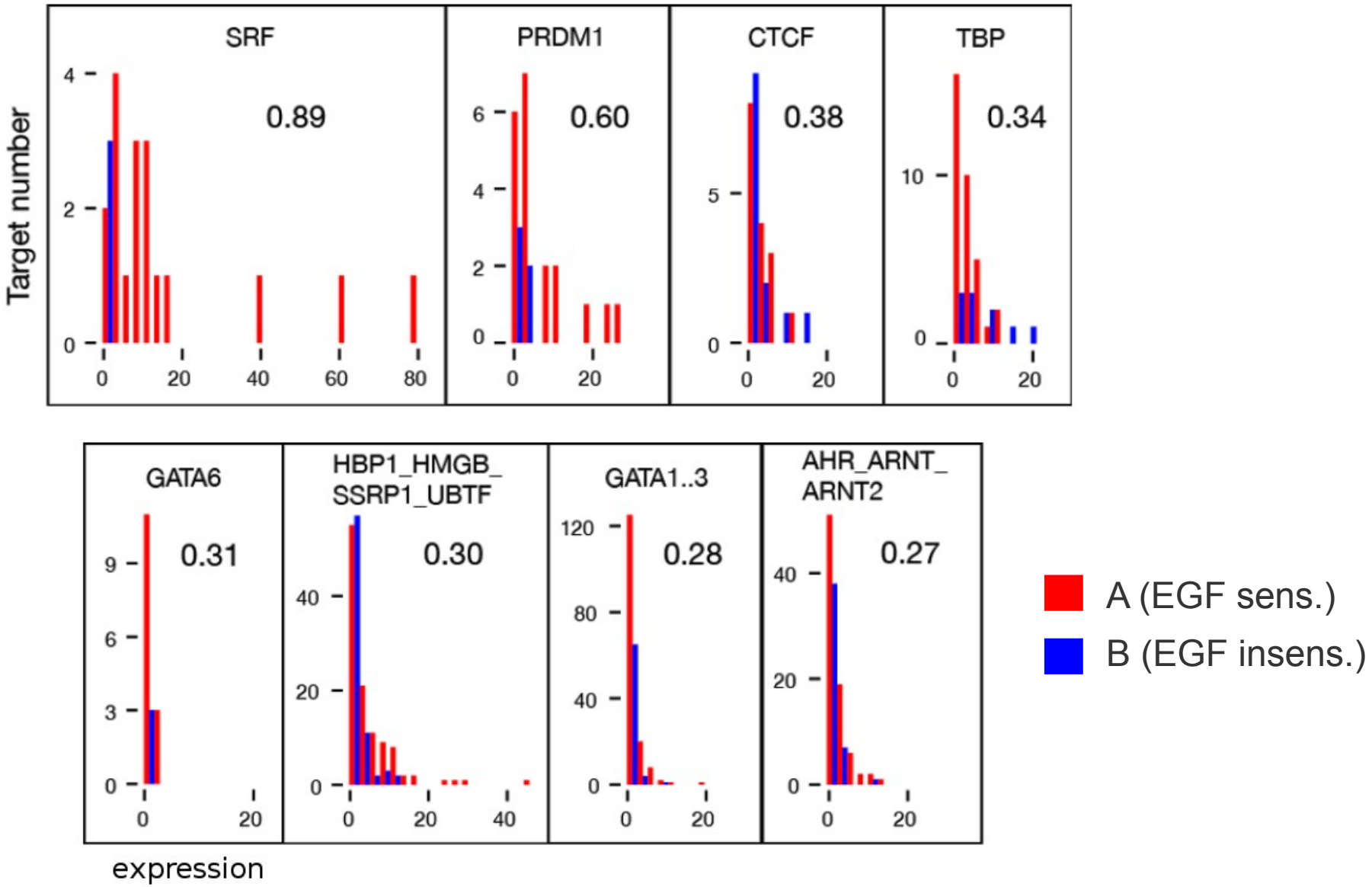
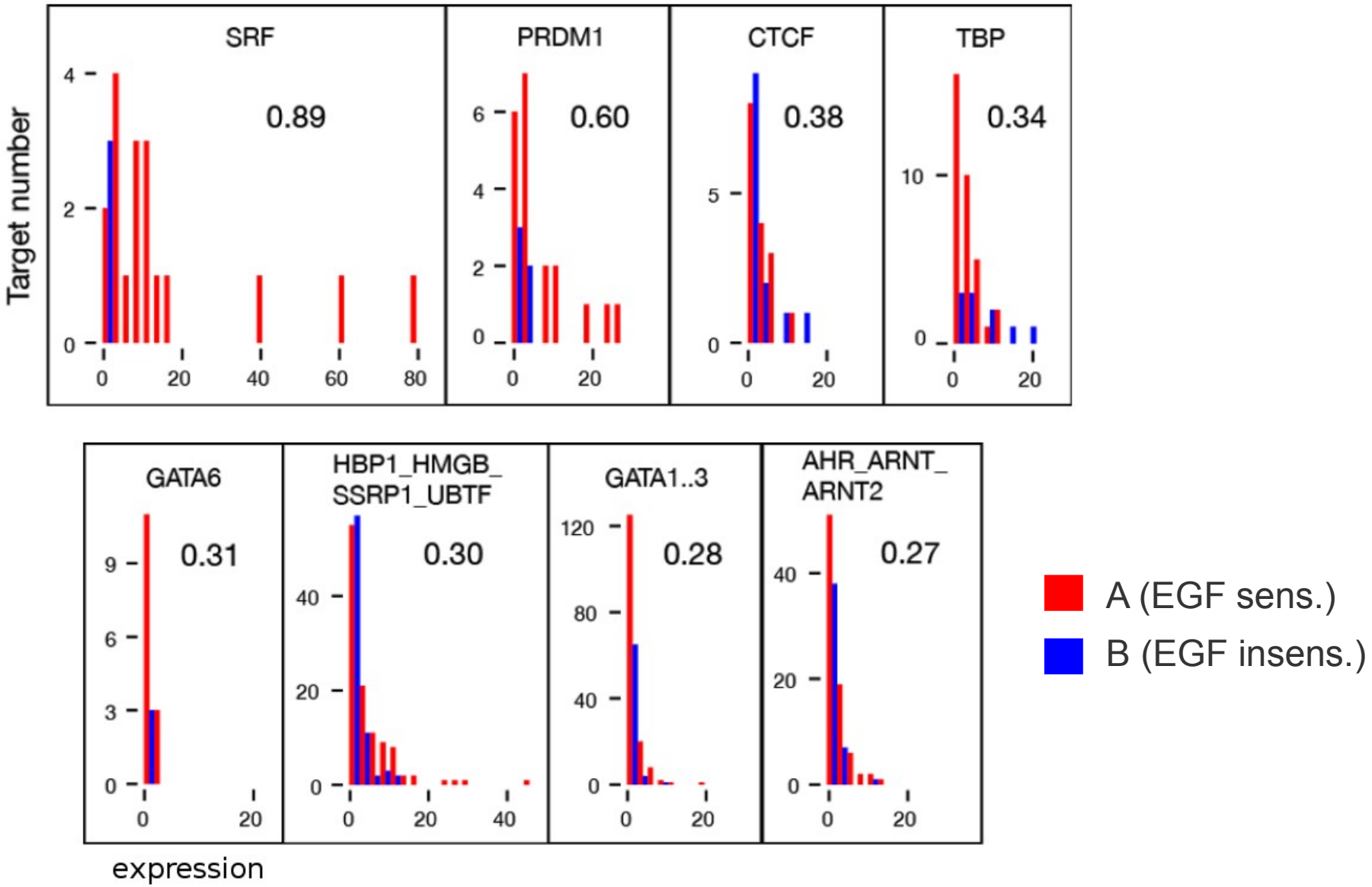

SRF
0.89
PRDM1
0.60
CTCF
0.38
TBP
0.34
Target number
GATA6
0.31
HBP1_HMGB_
SSRP1_UBTF
0.30
GATA1..3
0.28
AHR_ARNT_
ARNT2
0.27
expression
A (EGF sens.)
B (EGF insens.)

known new



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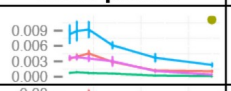
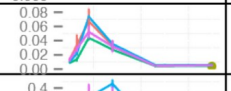
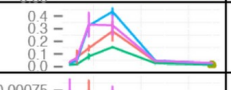
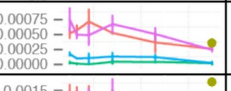
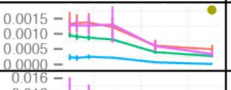
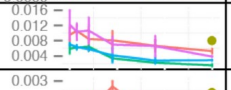
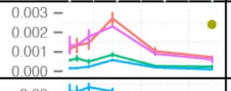
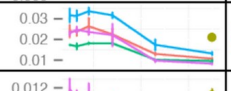
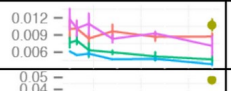
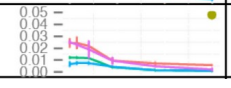
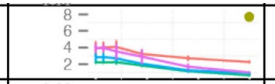
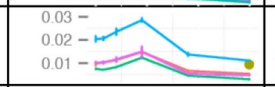
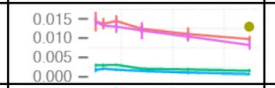
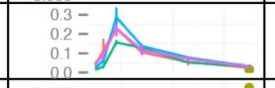
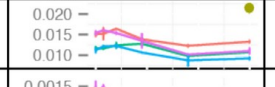
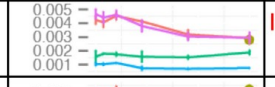
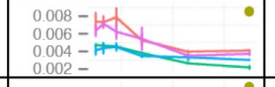
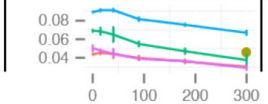
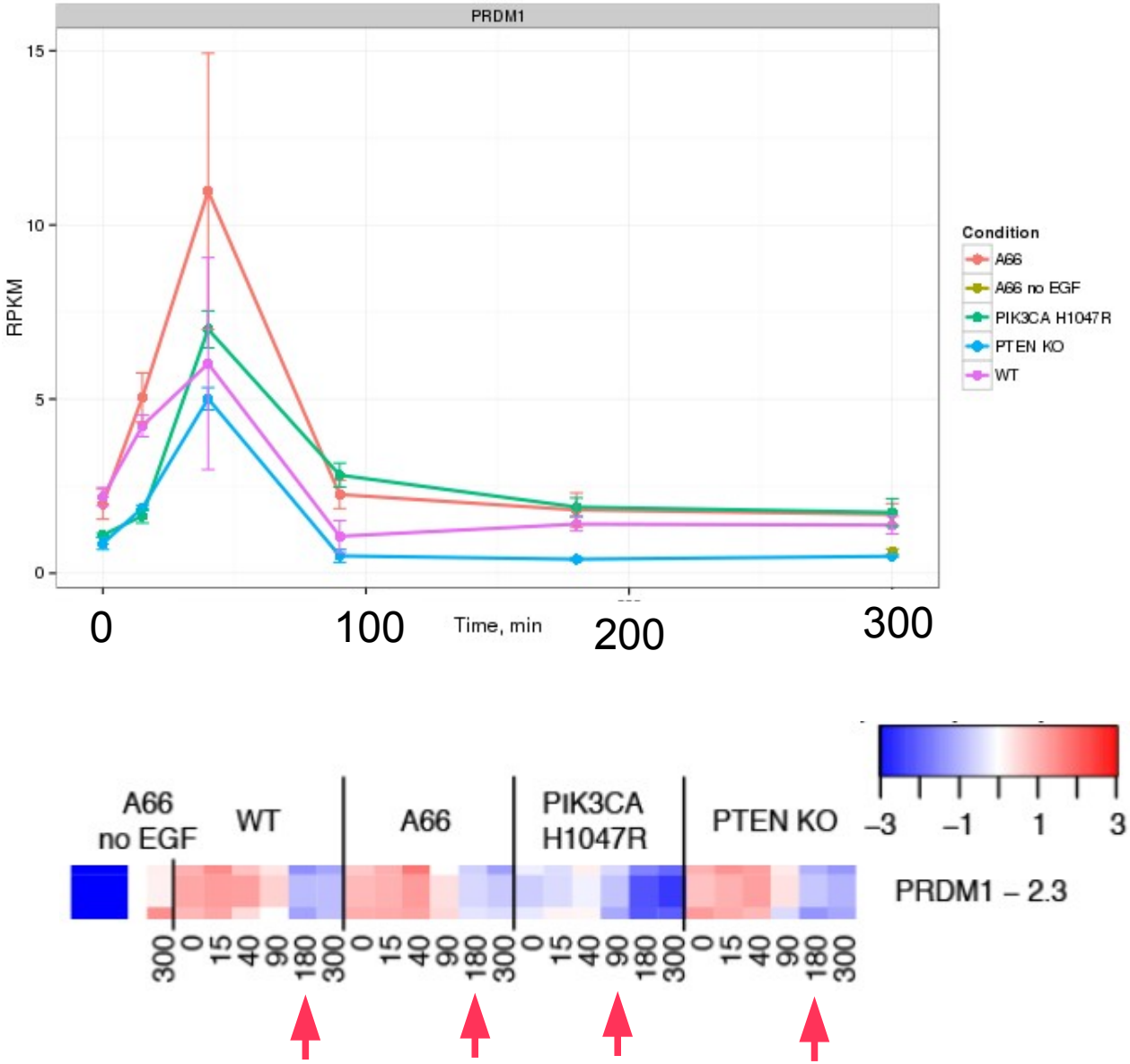
BLIMP1 (PRDM1) targets

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)

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		

EGF → PRDM1 expression
PIP3 → BLIMP1 function



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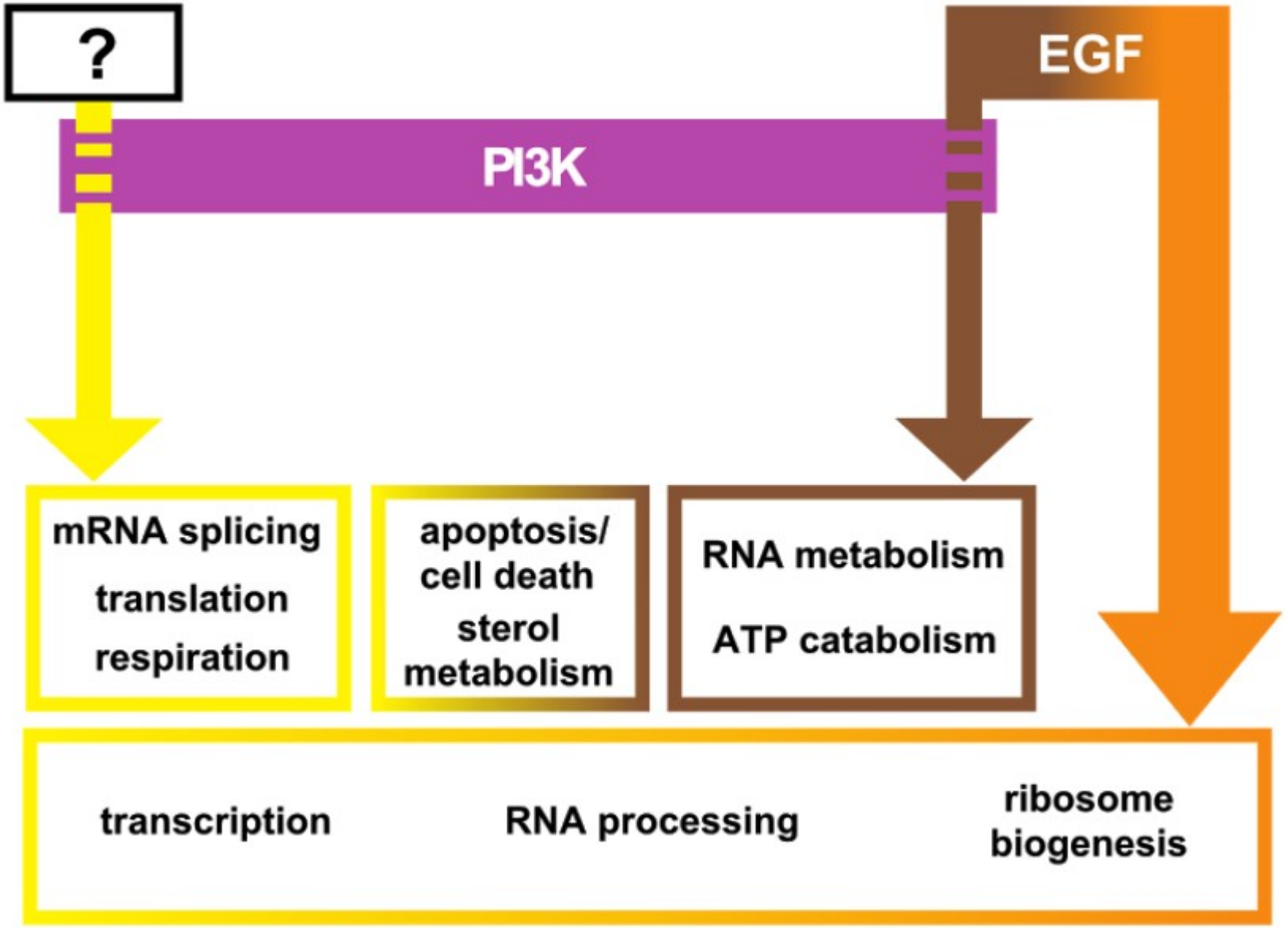
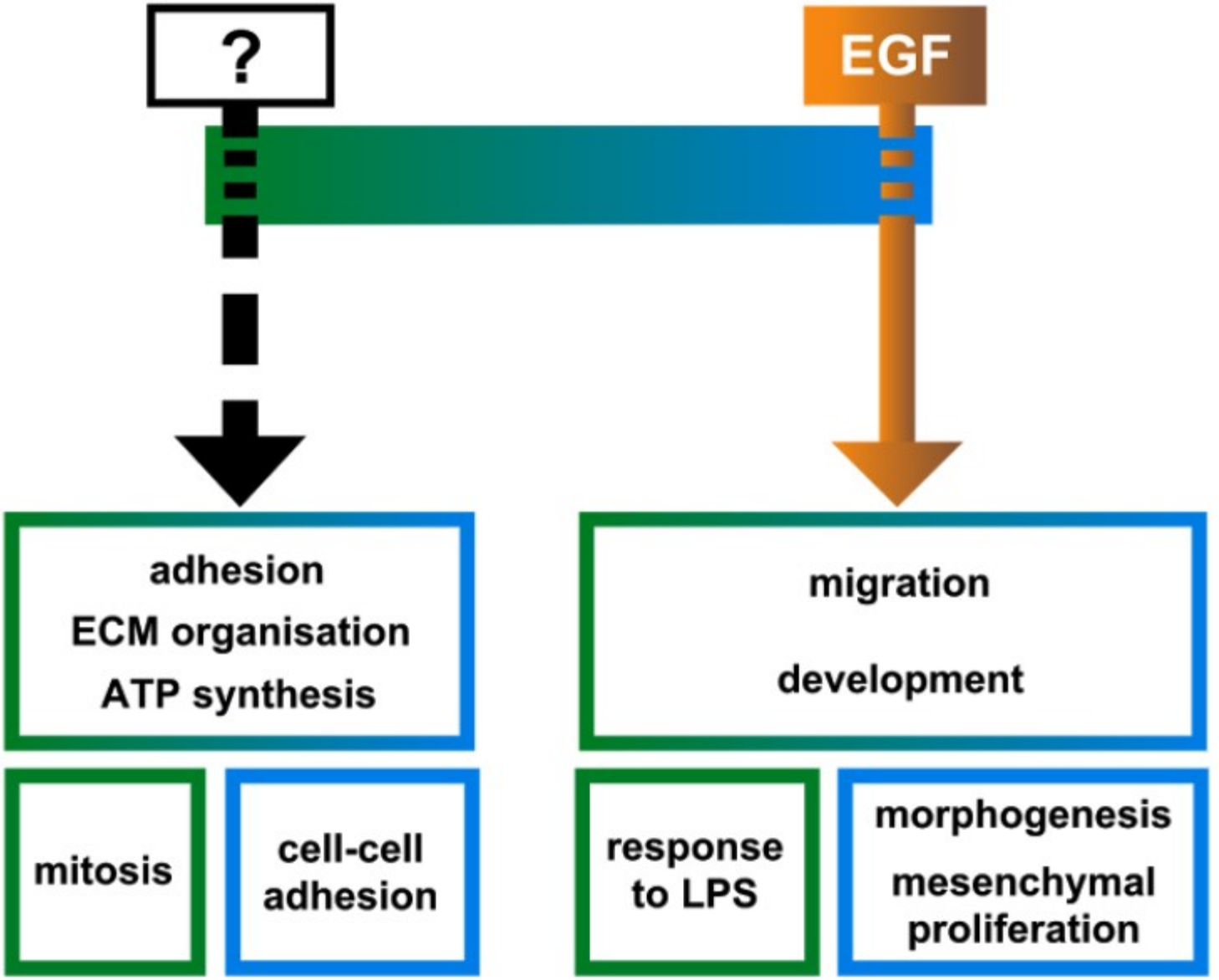
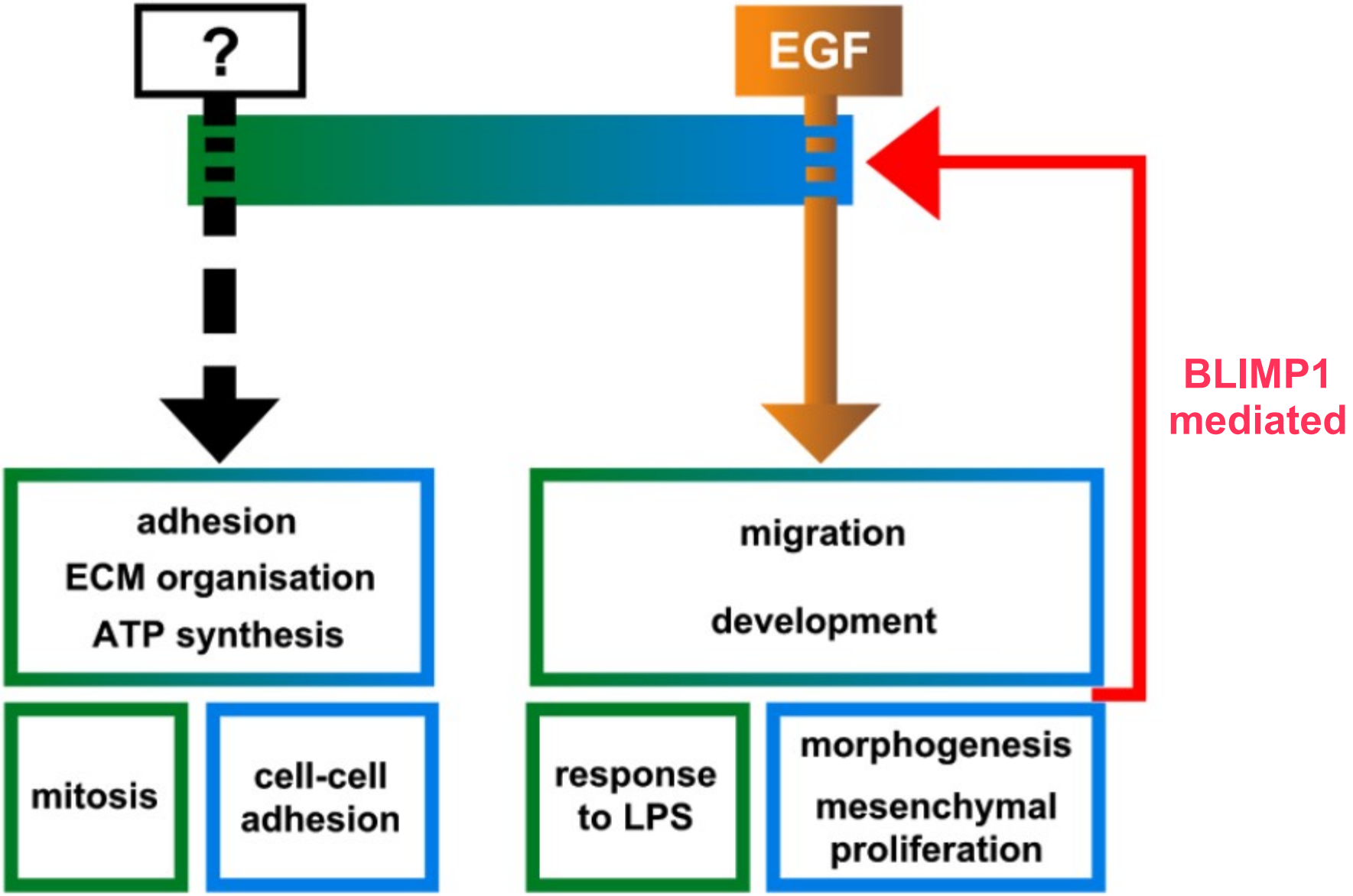
Acute perturbation of PIP3 signalling



Chronic perturbation of PIP3 signalling



Chronic perturbation of PIP3 signalling



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 (swarm of butterfly)

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

Now @ Sanger

EMBL-EBI

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Bioinformatics team

Simon Andrews
Anne Segond-Pichon

BBSRC bioscience for the future

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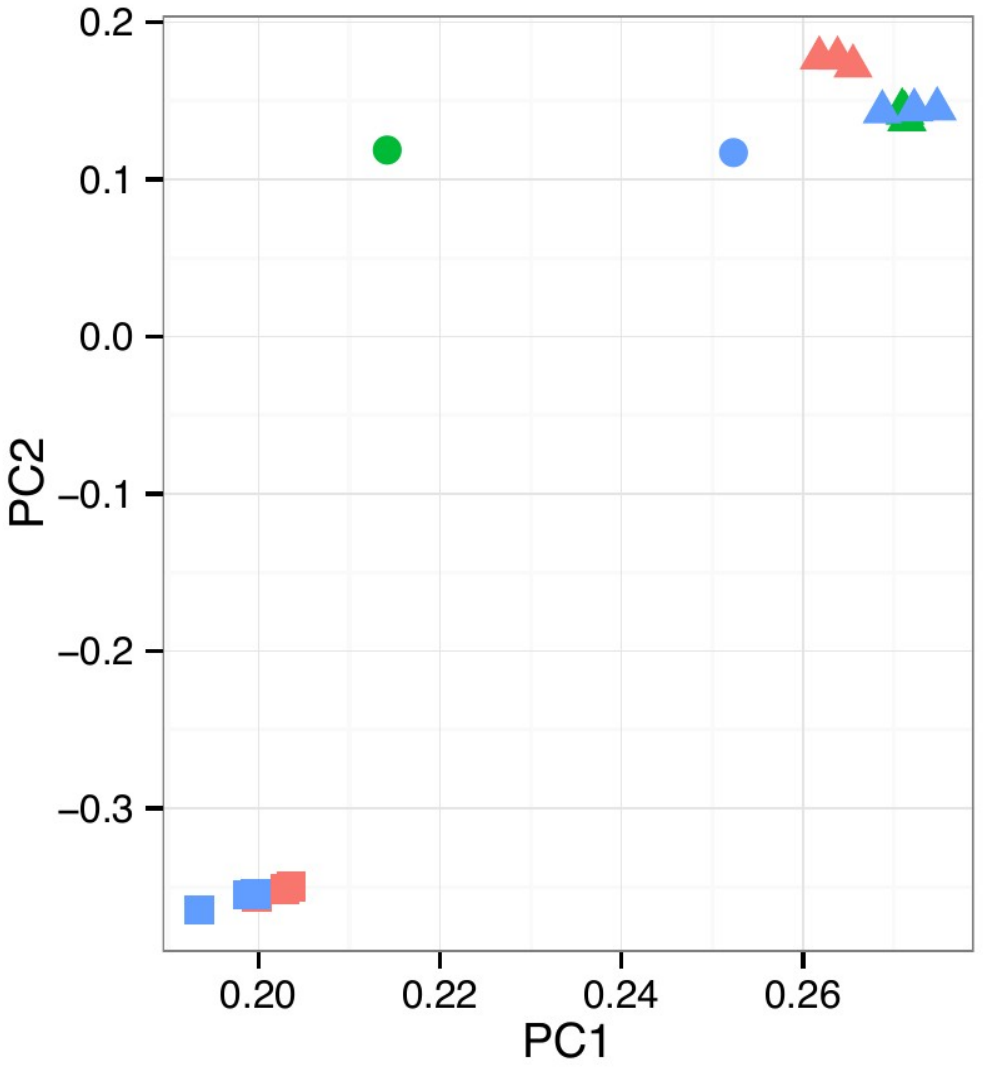
Biggest source of variability is the lab ...



Paper

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

Condition

- H1047R
- PTEN–/–
- WT

All cells are isogenic MCF10a

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