What can we Measure Today, Tomorrow and Which Experiments are Useful for Model Inference, and a Couple of (hopefully) Nice Examples

Dynamic Biological Modeling: Abstractions, Algorithms & Logic

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August11th, 2015

The Cell as an Information Processing System

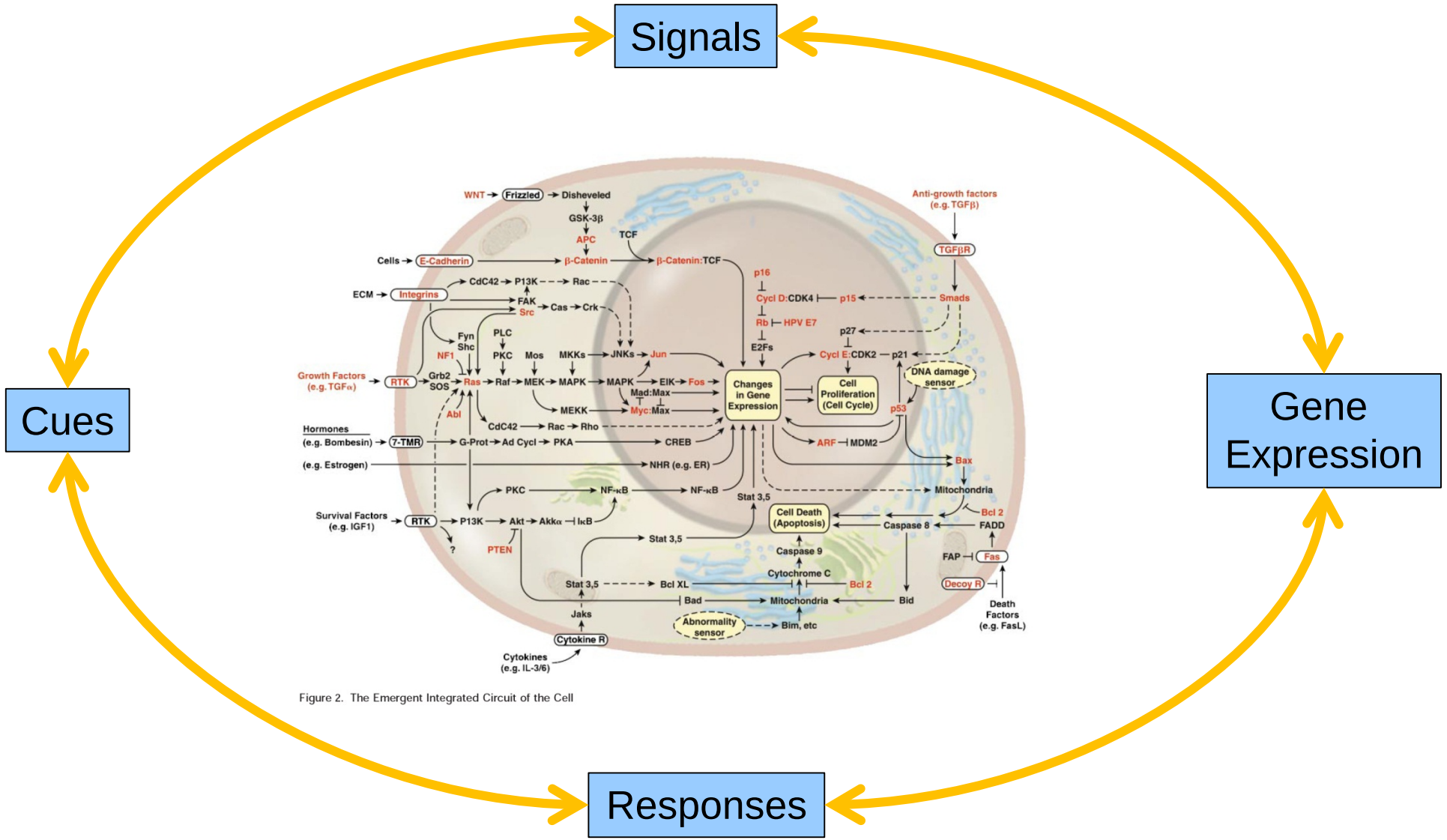
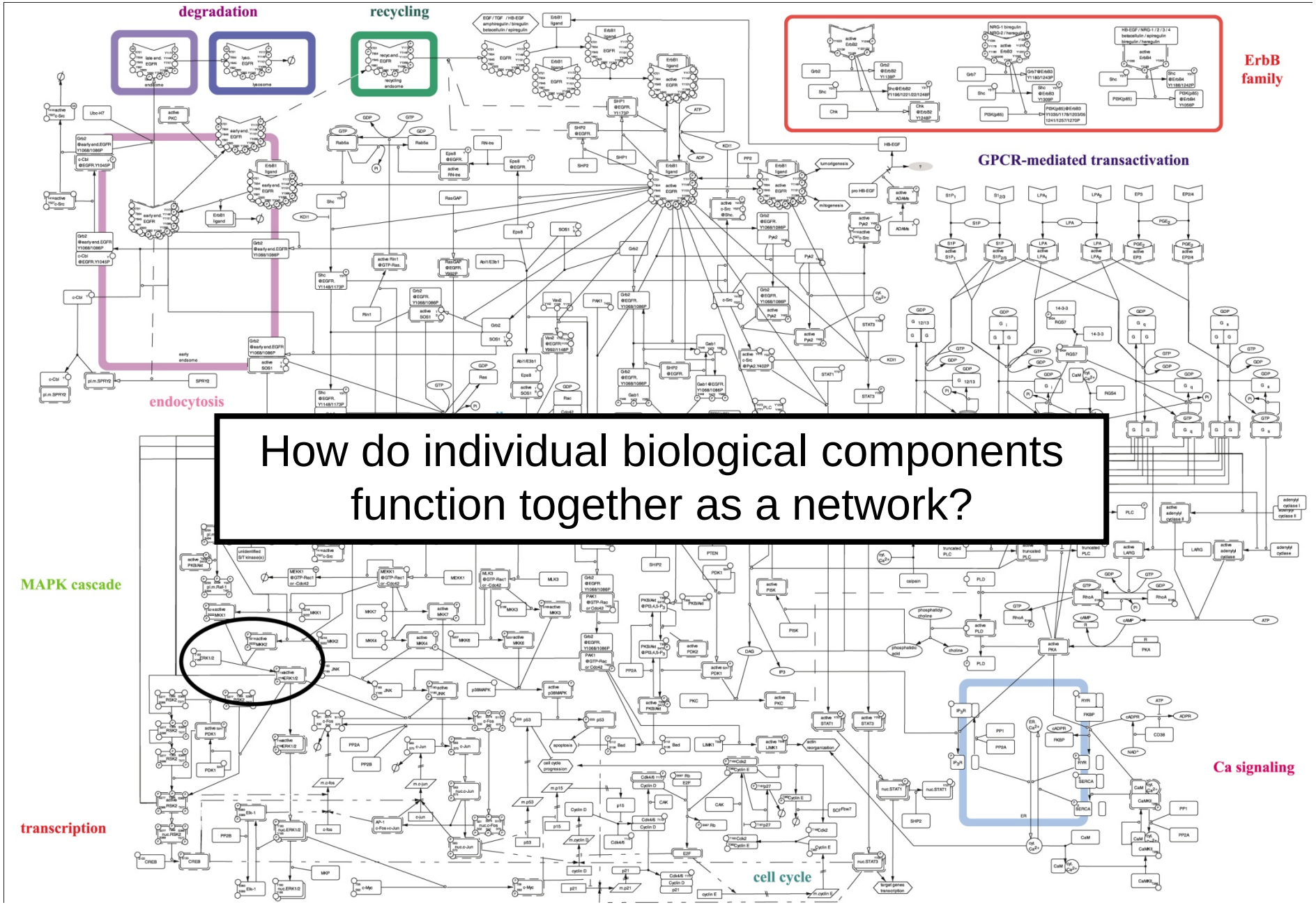


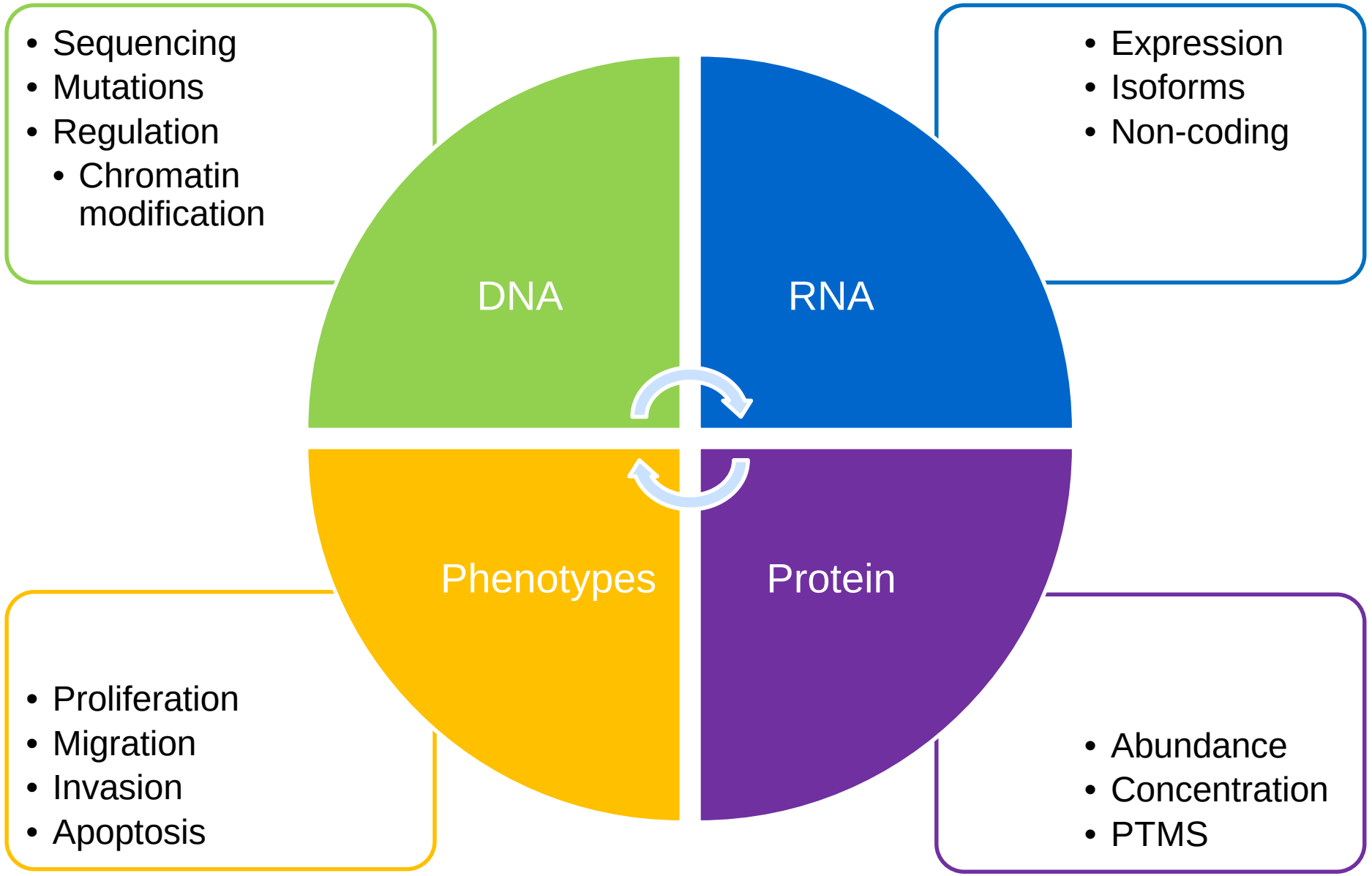
Figure 2. The Emergent Integrated Circuit of the Cell

From Hanahan & Weinberg (2000) Cell 100: 57-90

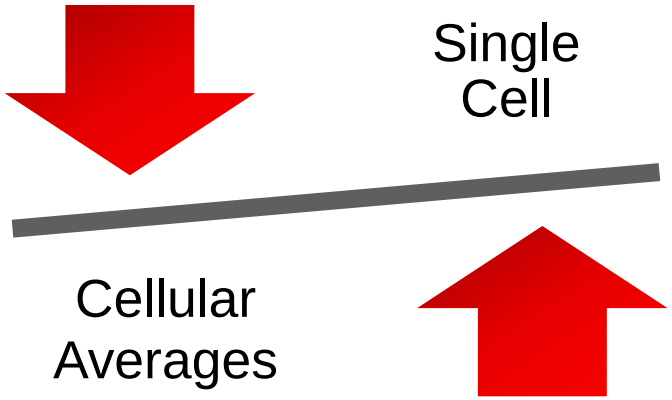


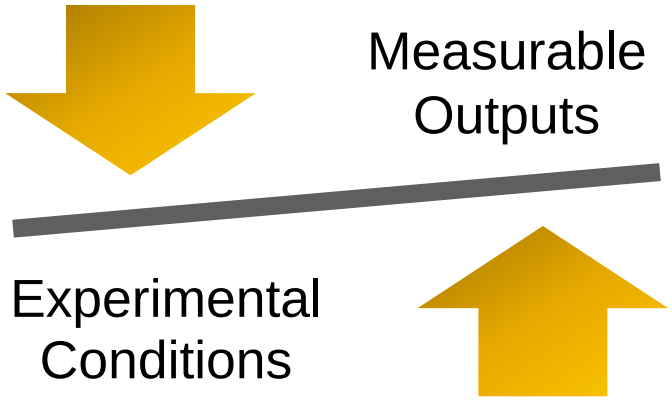
Oda *et al.* **Mol Syst. Biol.** (2005)

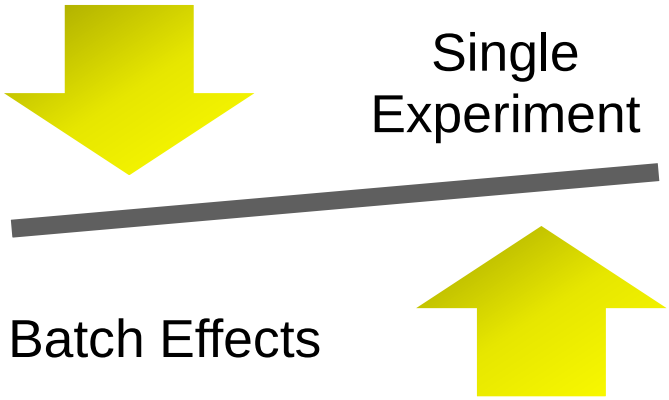
What can we measure?

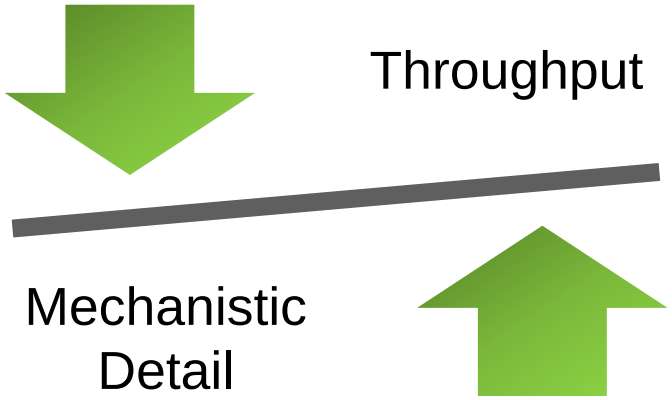


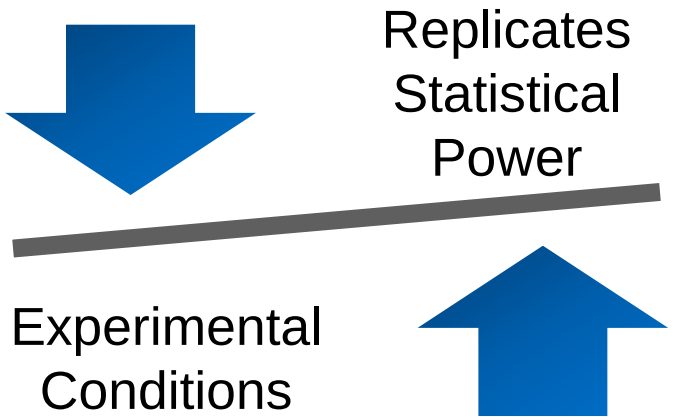
Common Trade offs

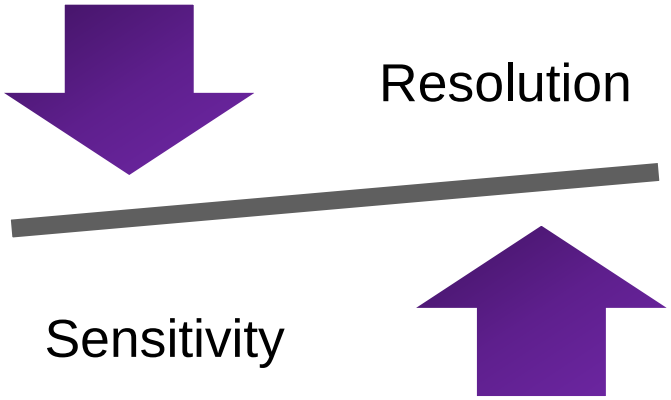






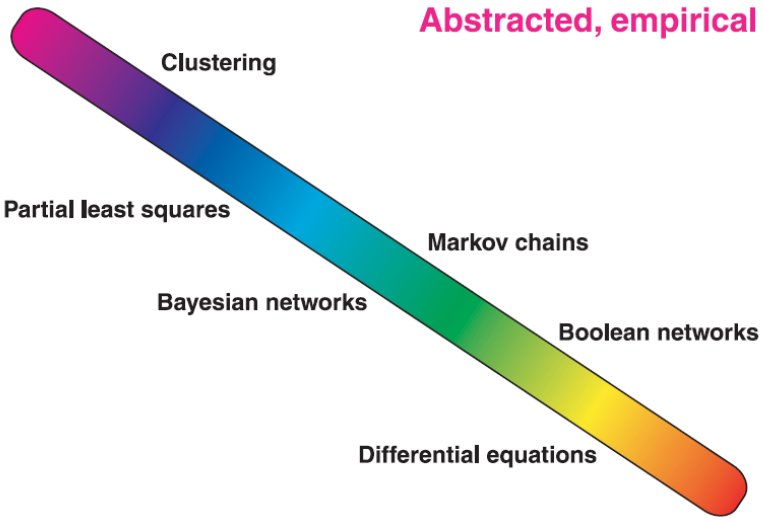


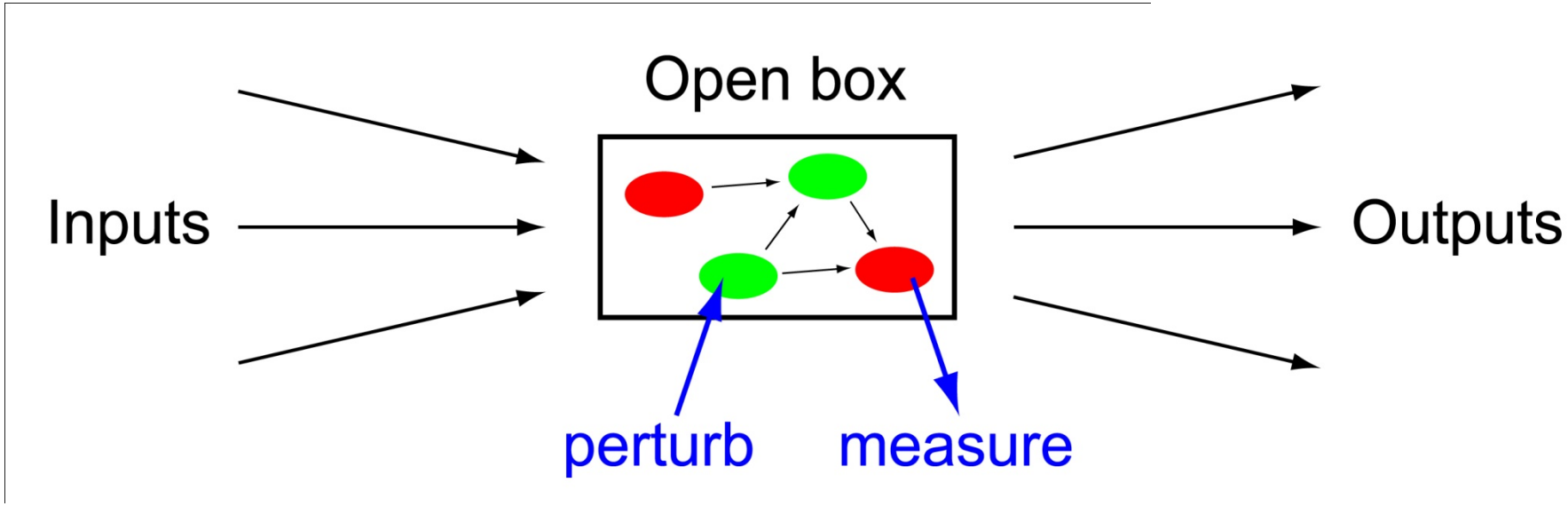




Not all experiments/models are created equally

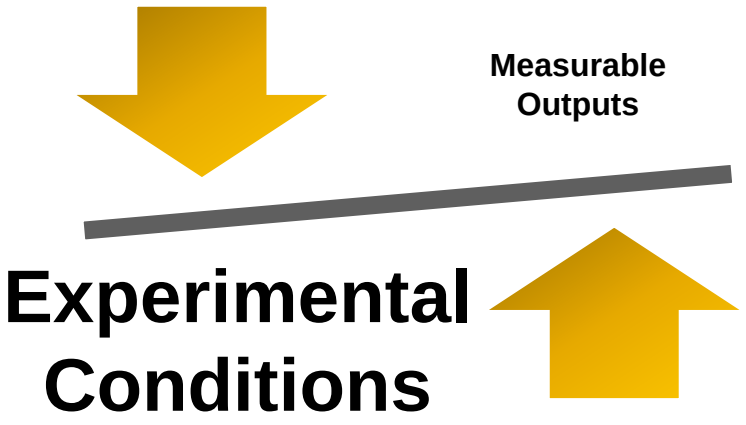
- Talk to your colleagues
  - What model approach fits the problem
- Compare conditions / Perturbations

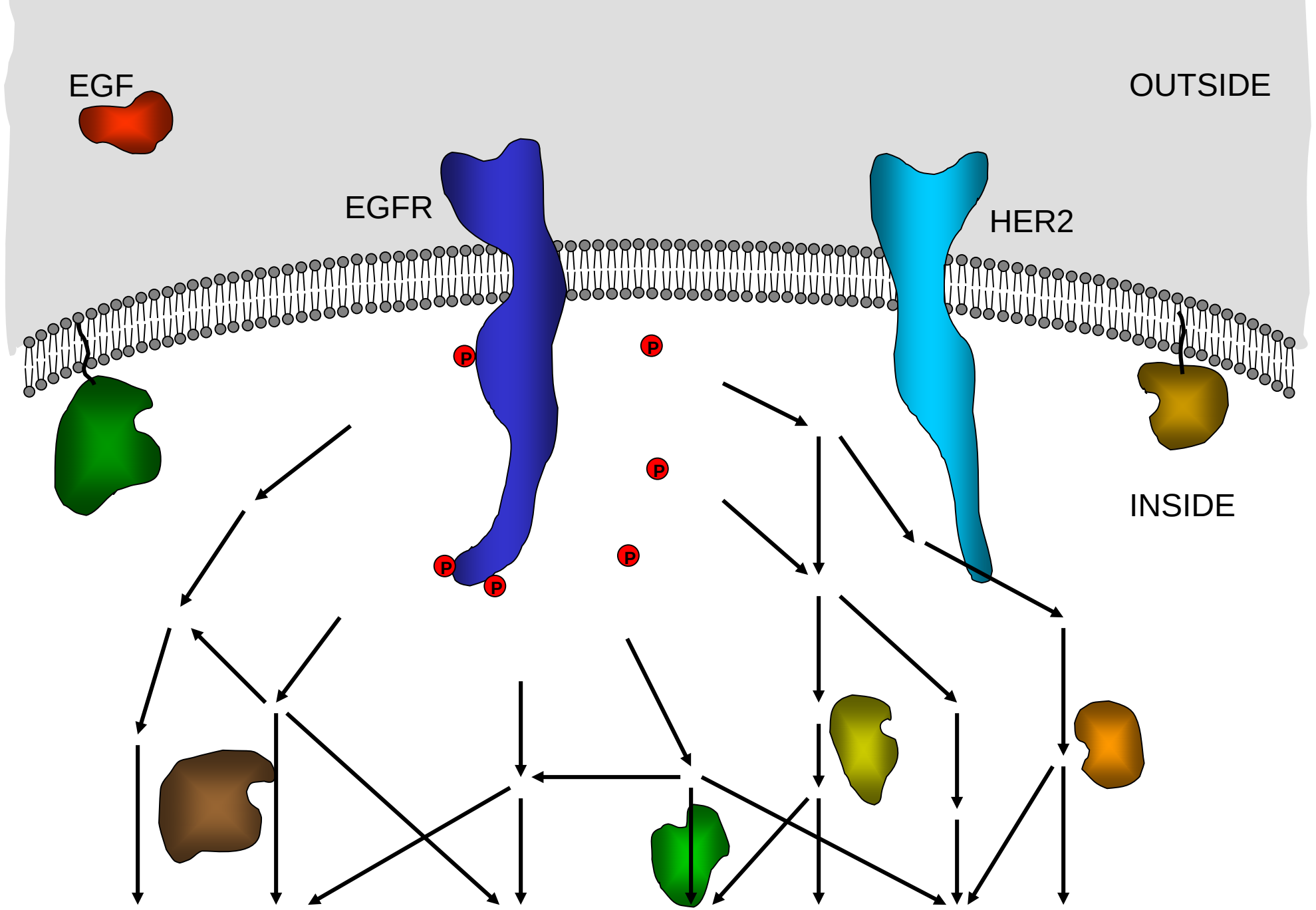




Case Study One: Receptor Tyrosine Kinases

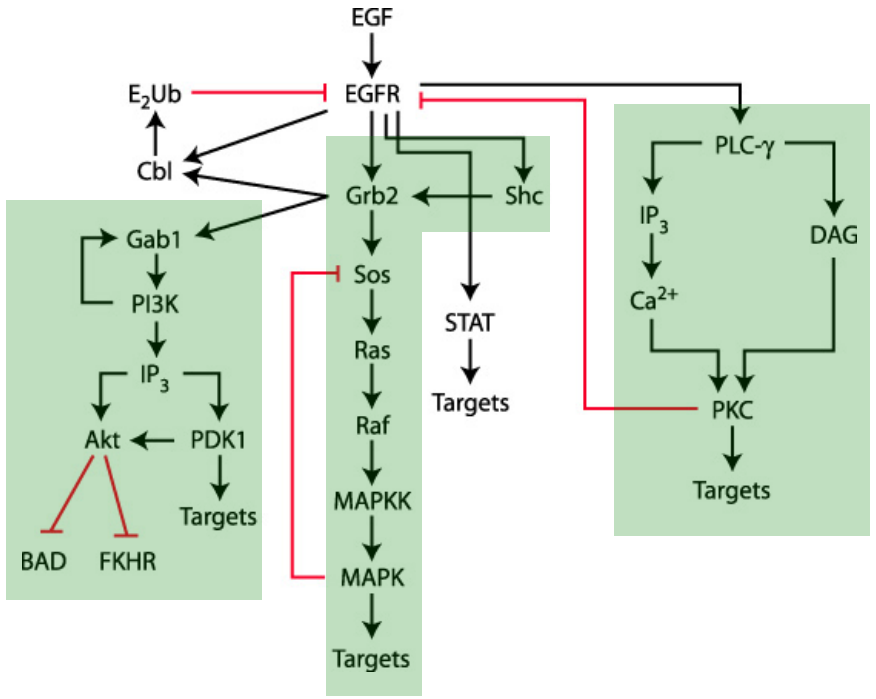
- Determinants of phenotype:
  - Proliferation
  - Migration/Invasio
  - Differentiation
- Deregulation leads to disease
  - Cancer
  - Diabetes
  - Alzheimer’s



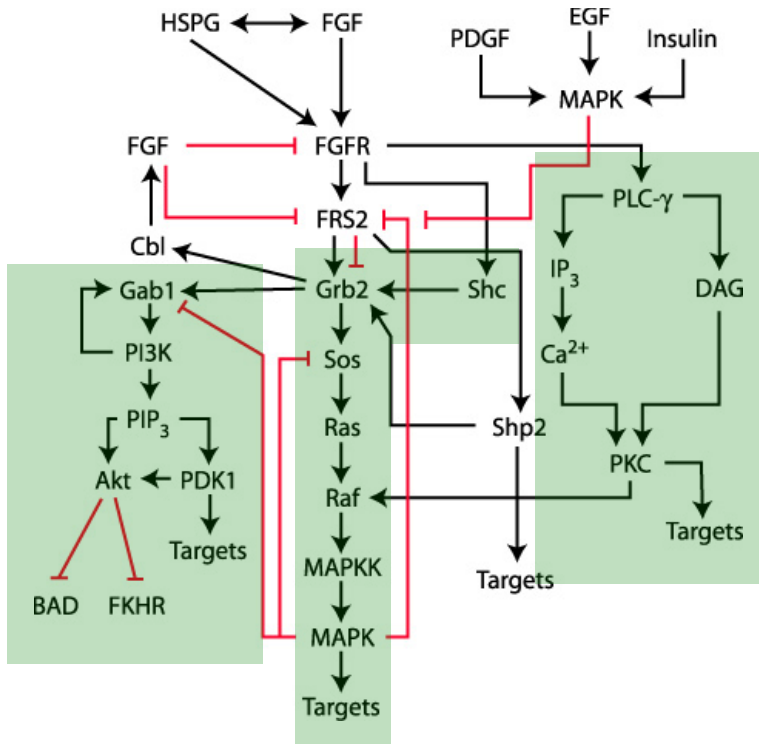

EGF
OUTSIDE
EGFR
HER2
INSIDE
P
P
P
P
P

Binary Wiring Diagrams are Remarkably Similar for Different RTKs

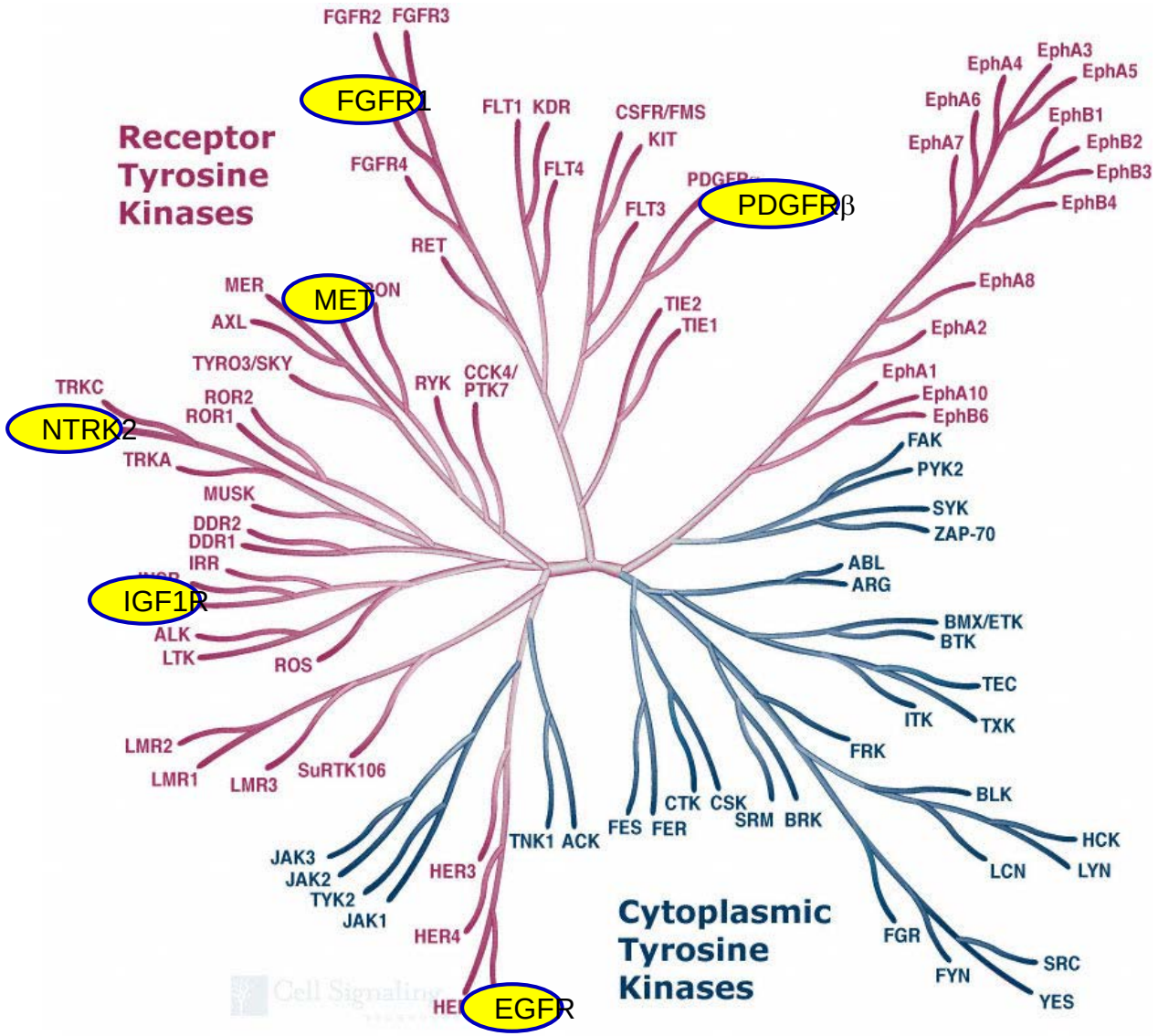
EGFR



FGFR1



Schlessinger Science 2004


Receptor
Tyrosine
Kinases
FGFR2
FGFR3
FGFR1
FGFR4
FLT1
KDR
CSFR/FMS
KIT
FLT4
FLT3
PDGFRβ
RET
MER
MET
AXL
TYRO3/SKY
RYK
CCK4/
PTK7
TIE2
TIE1
TRKC
ROR2
ROR1
NTRK2
TRKA
MUSK
DDR2
DDR1
IRR
IGF1R
ALK
LTK
ROS
LMR2
LMR1
LMR3
SuRTK106
EphA4
EphA3
EphA5
EphA6
EphB1
EphA7
EphB2
EphB3
EphB4
EphA8
EphA2
EphA1
EphA10
EphB6
FAK
PYK2
SYK
ZAP-70
ABL
ARG
BMX/ETK
BTK
TEC
ITK
TXK
FRK
BLK
HCK
LCN
LYN
FGR
FYN
SRC
YES
Cytoplasmic
Tyrosine
Kinases
JAK3
JAK2
TYK2
JAK1
HER3
HER4
HE
EGFR
TNK1
ACK
FES
FER
CTK
CSK
SRM
BRK

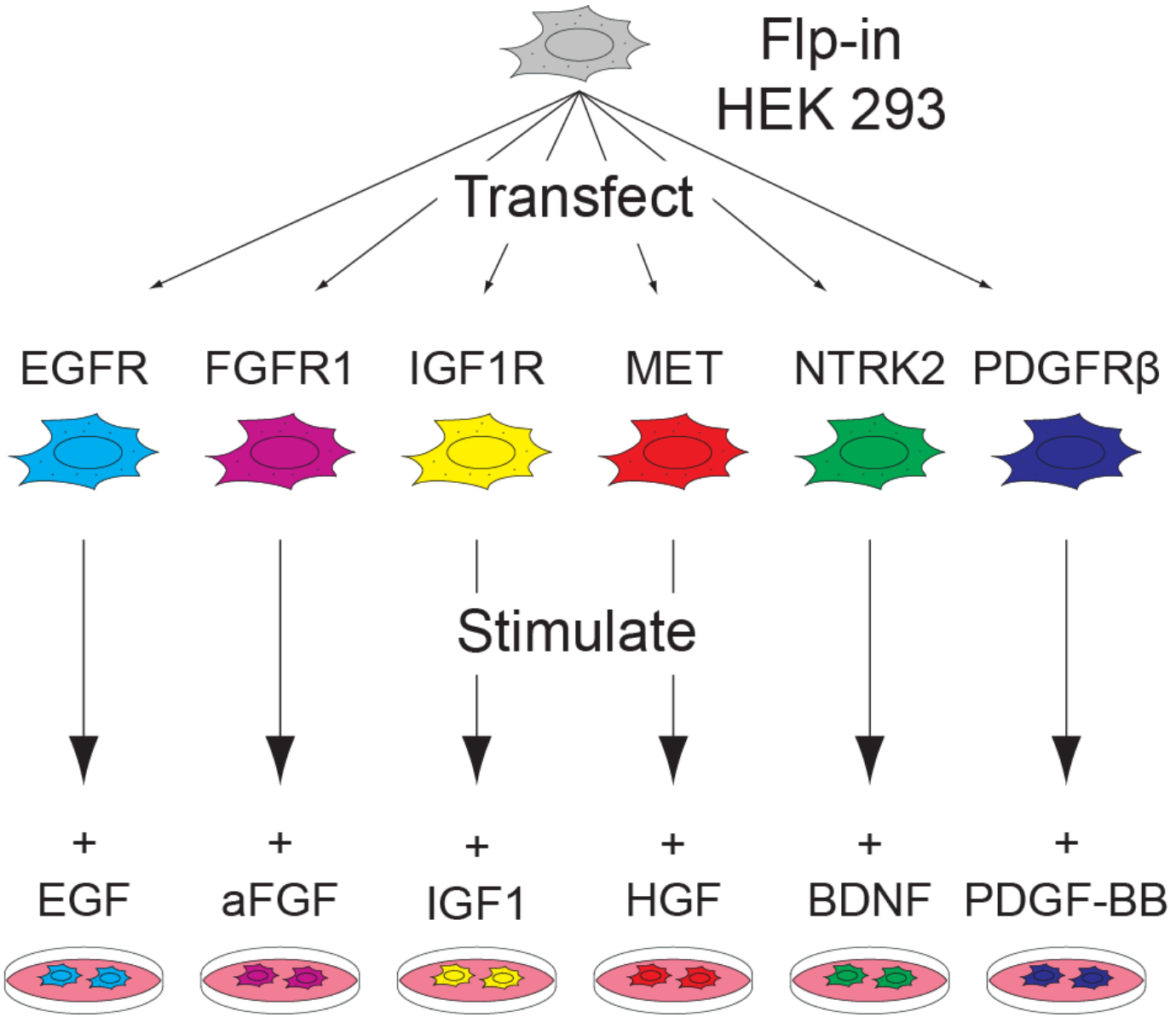
Overview

- We want to understand:
  - Network Connections
  - Network Dynamics
  - Relations among RTKs
  - Relations between RTKs and cancer

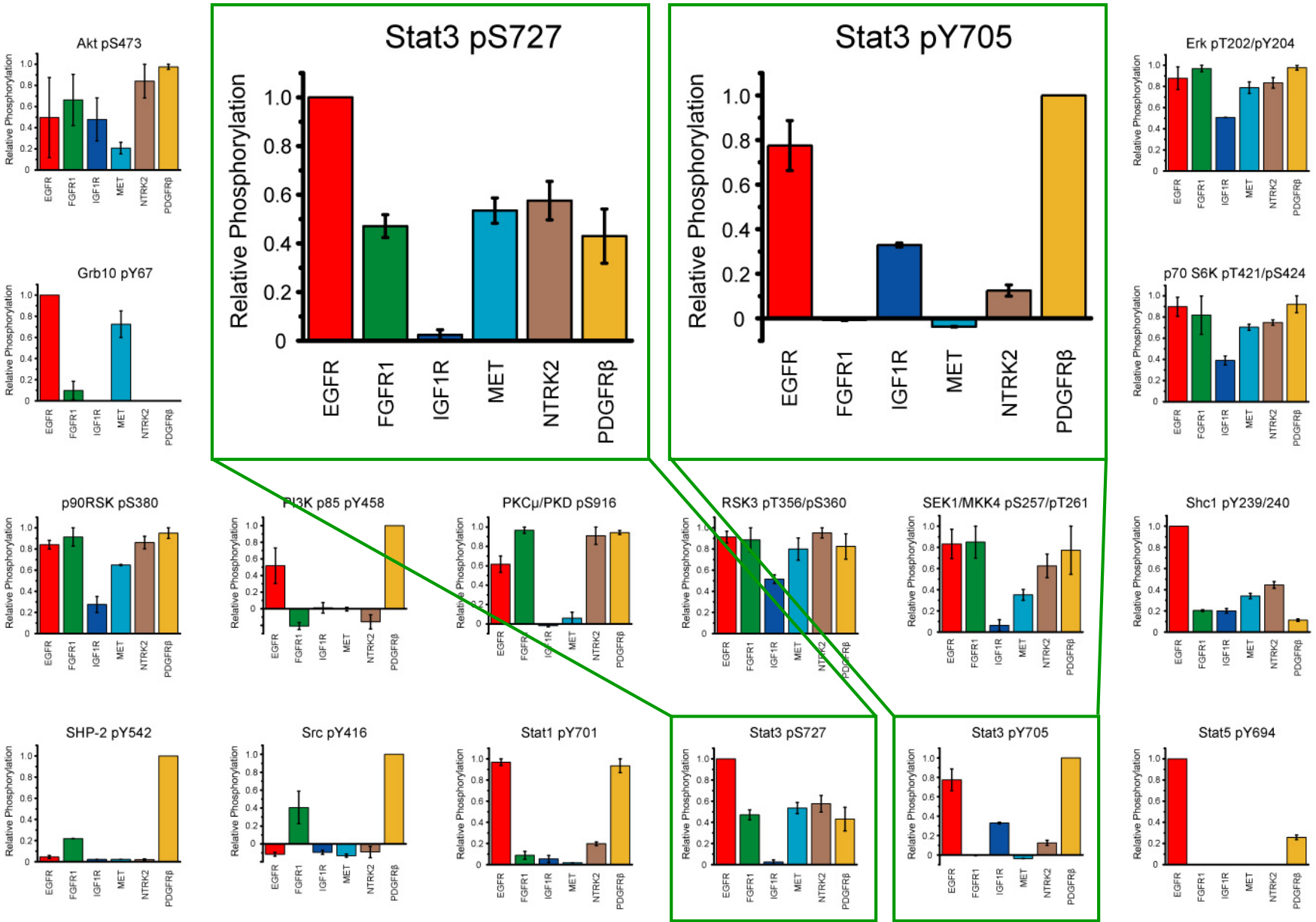
- For every RTK we need to:
  - Disrupt the network
  - Measure the network dynamics

- Thousands of samples to collect and measure

Cell Line Treatments

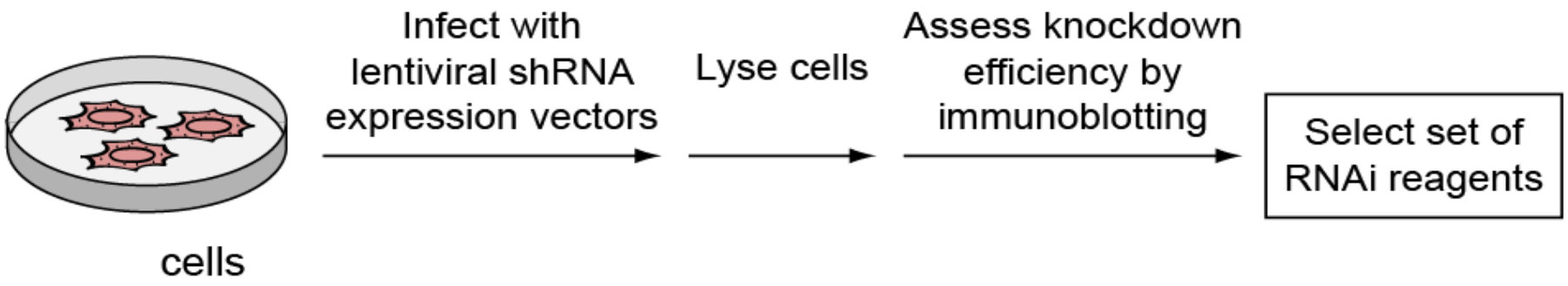


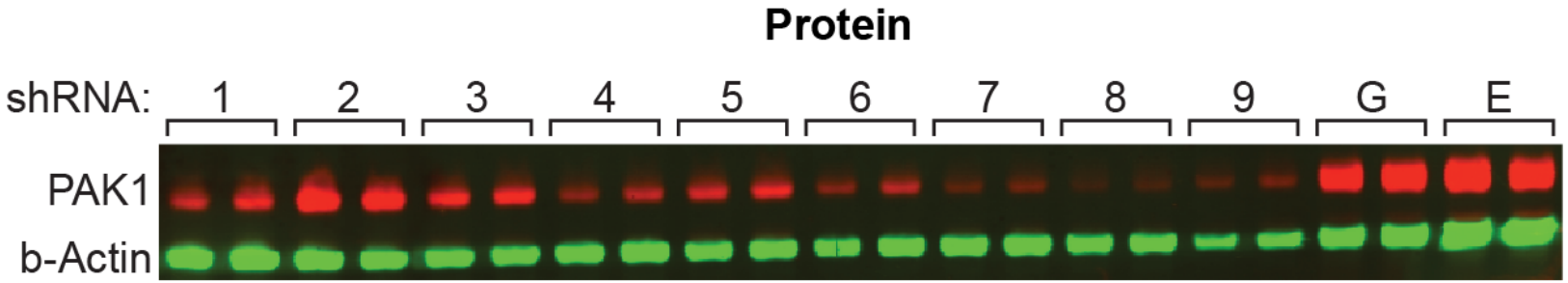
Different RTKs activate different signaling nodes



Cellular networks are disrupted using high quality shRNA reagents selected by immunoblotting

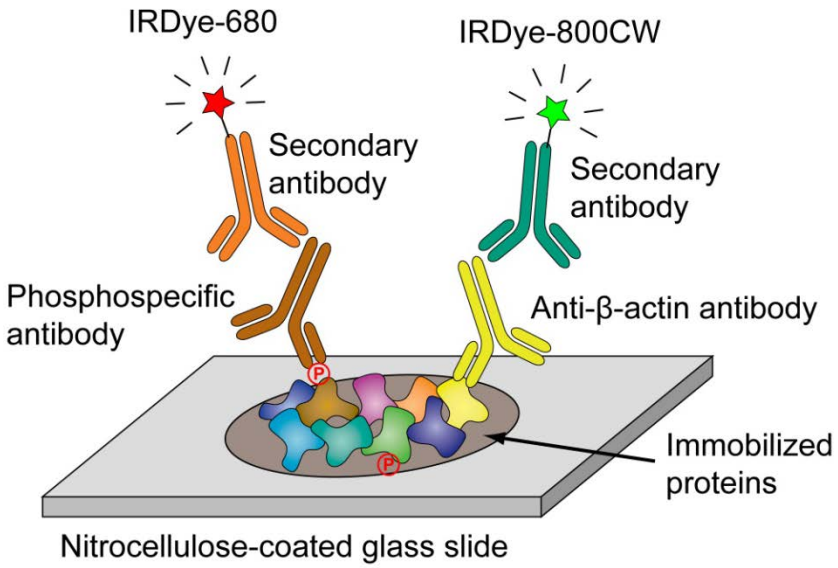
Second, we need to validate reagents that will allow us to consistently perturb different cell lines



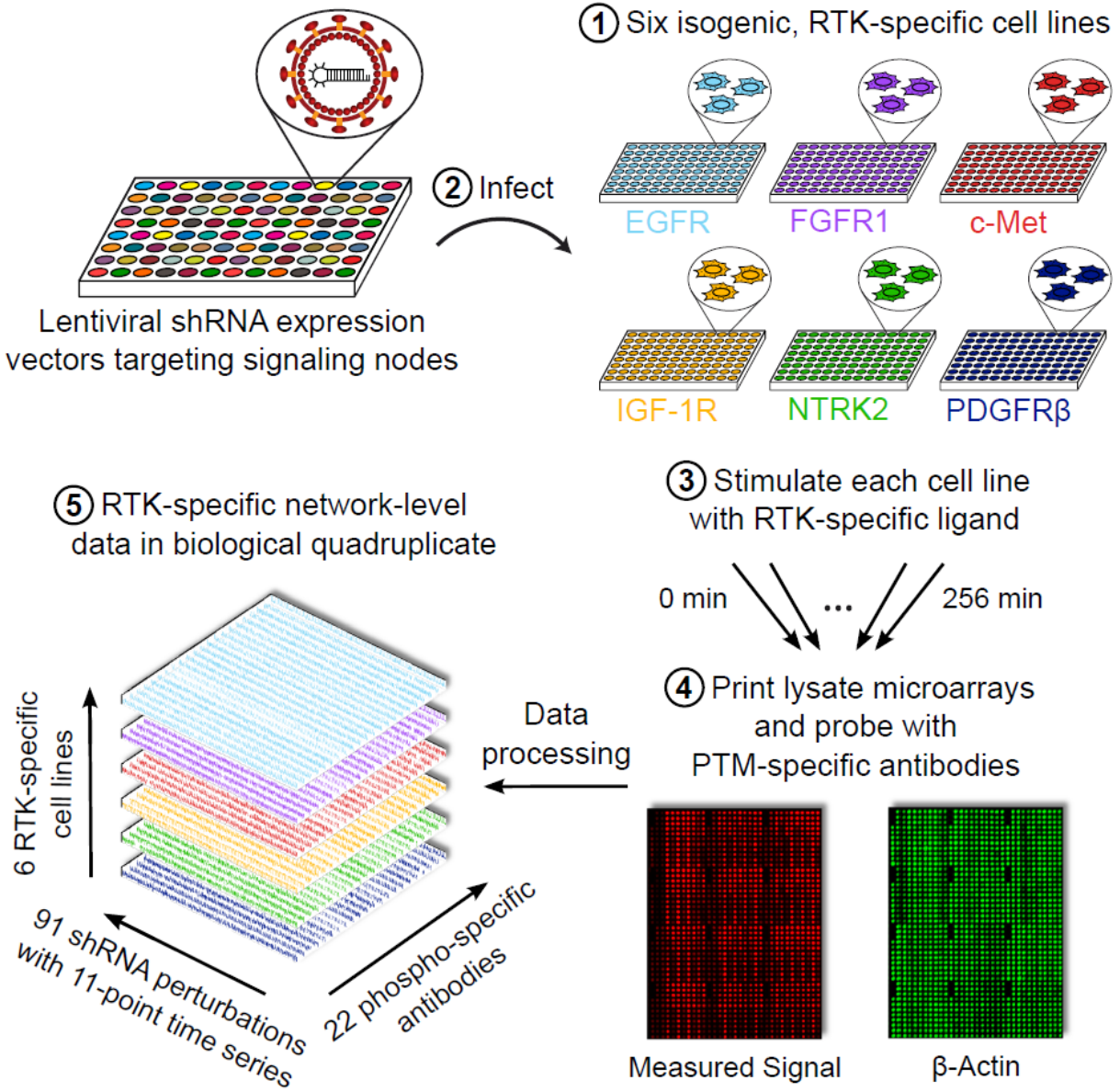


-Test Broad institute library of hairpins for selected signaling nodes
-Chose 2 with highest knockdown efficiency for future work

Detection Method

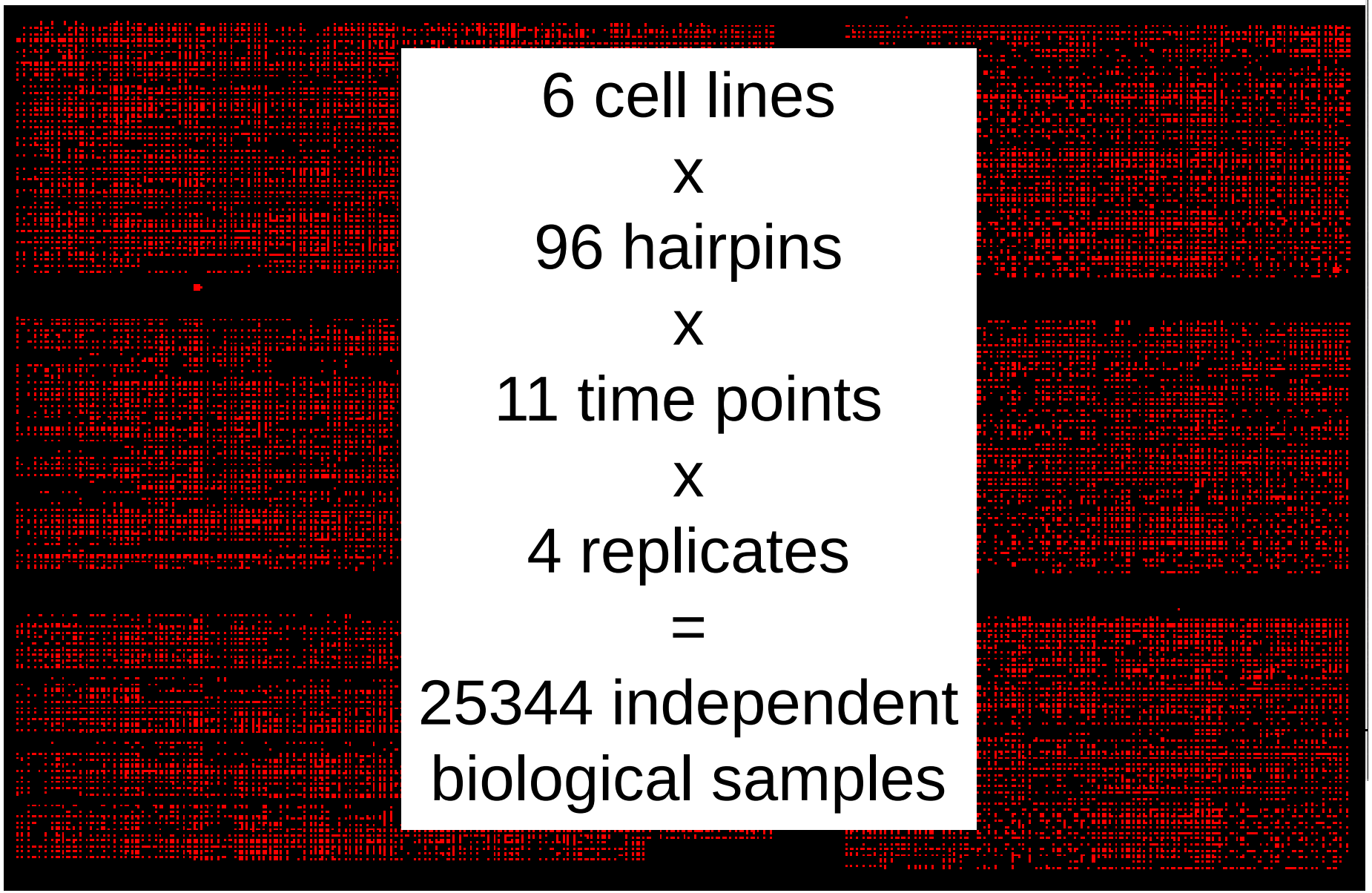


Experimental Design

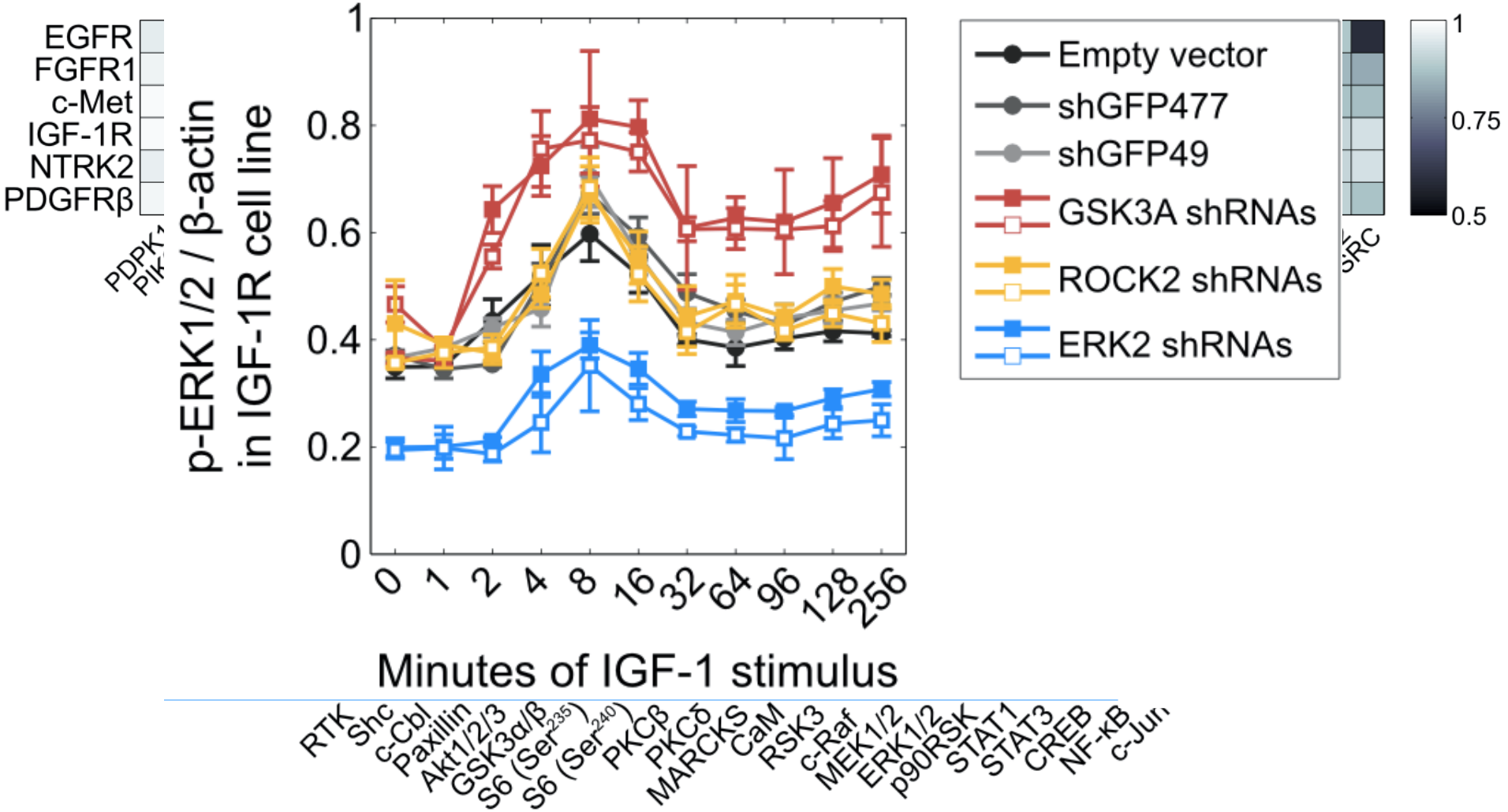


Wagner, Wolf-Yadlin et al. Science Signaling 2013

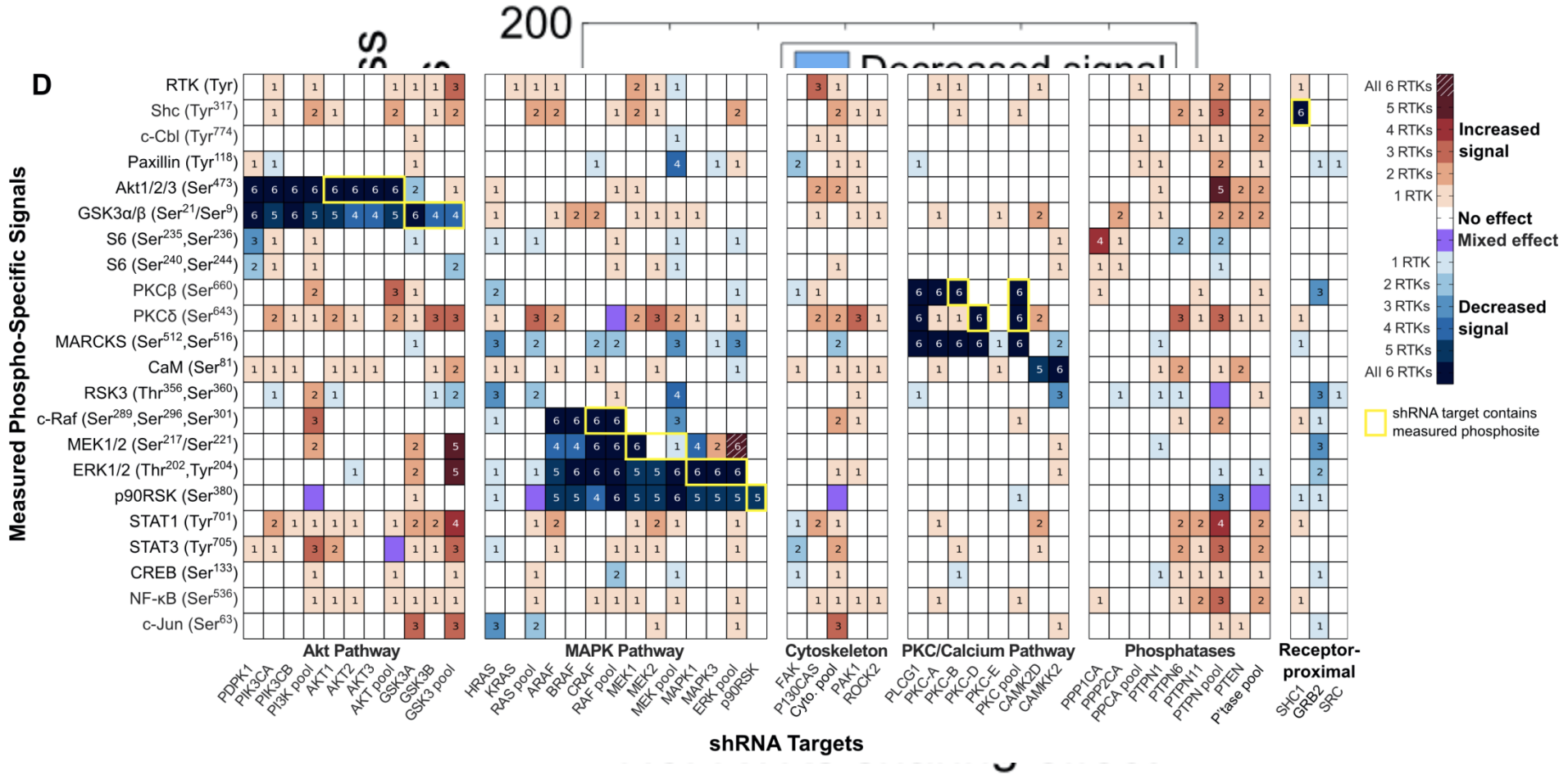
α-phospho-ERK (pT202/pY204)



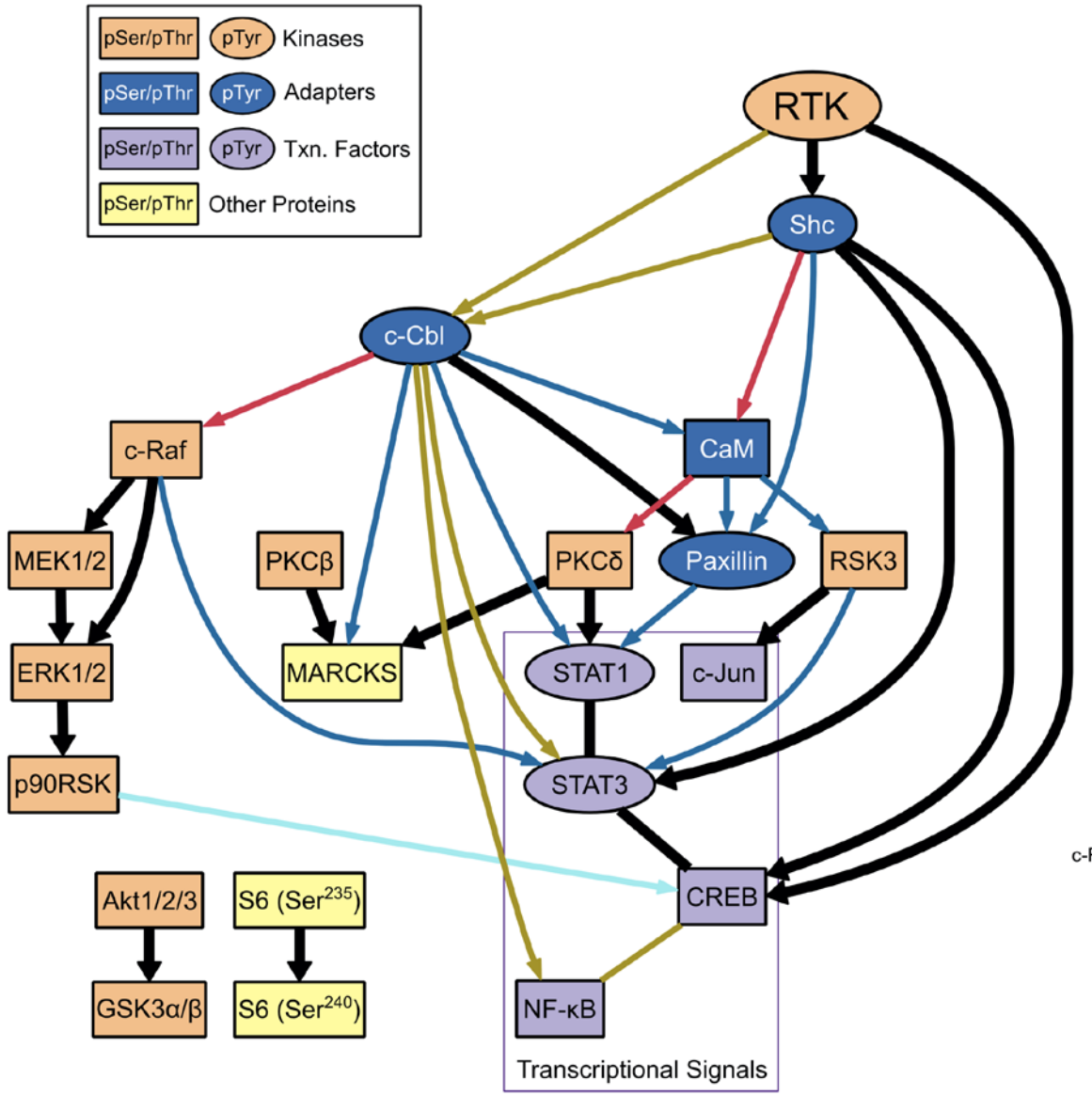
Perturbations reveal specificity in RTK-induced signal transduction

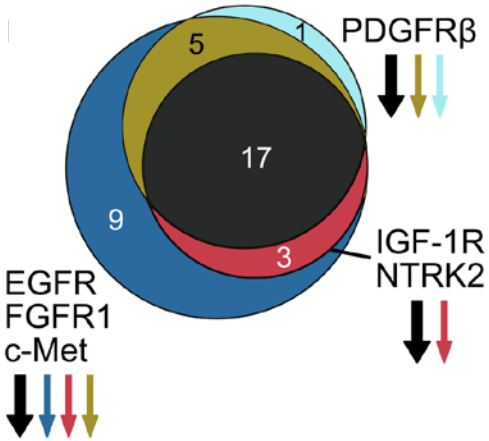


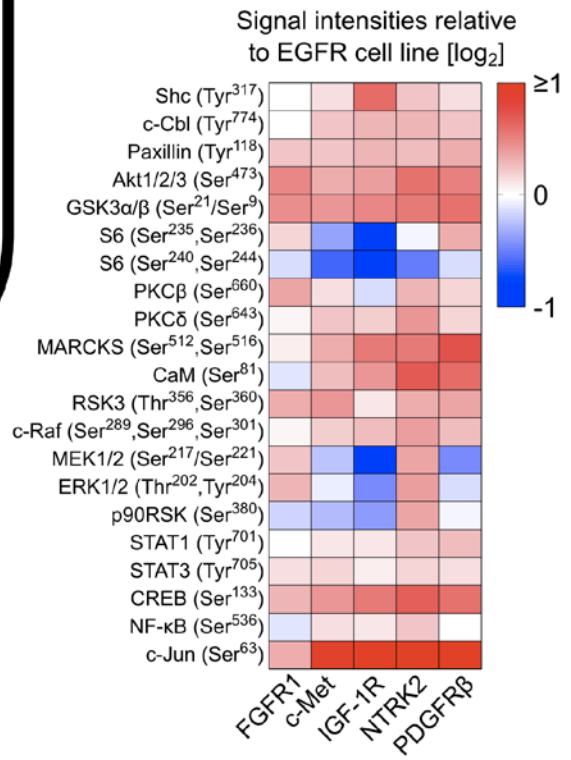
Perturbations reveal specificity in RTK-induced signal transduction



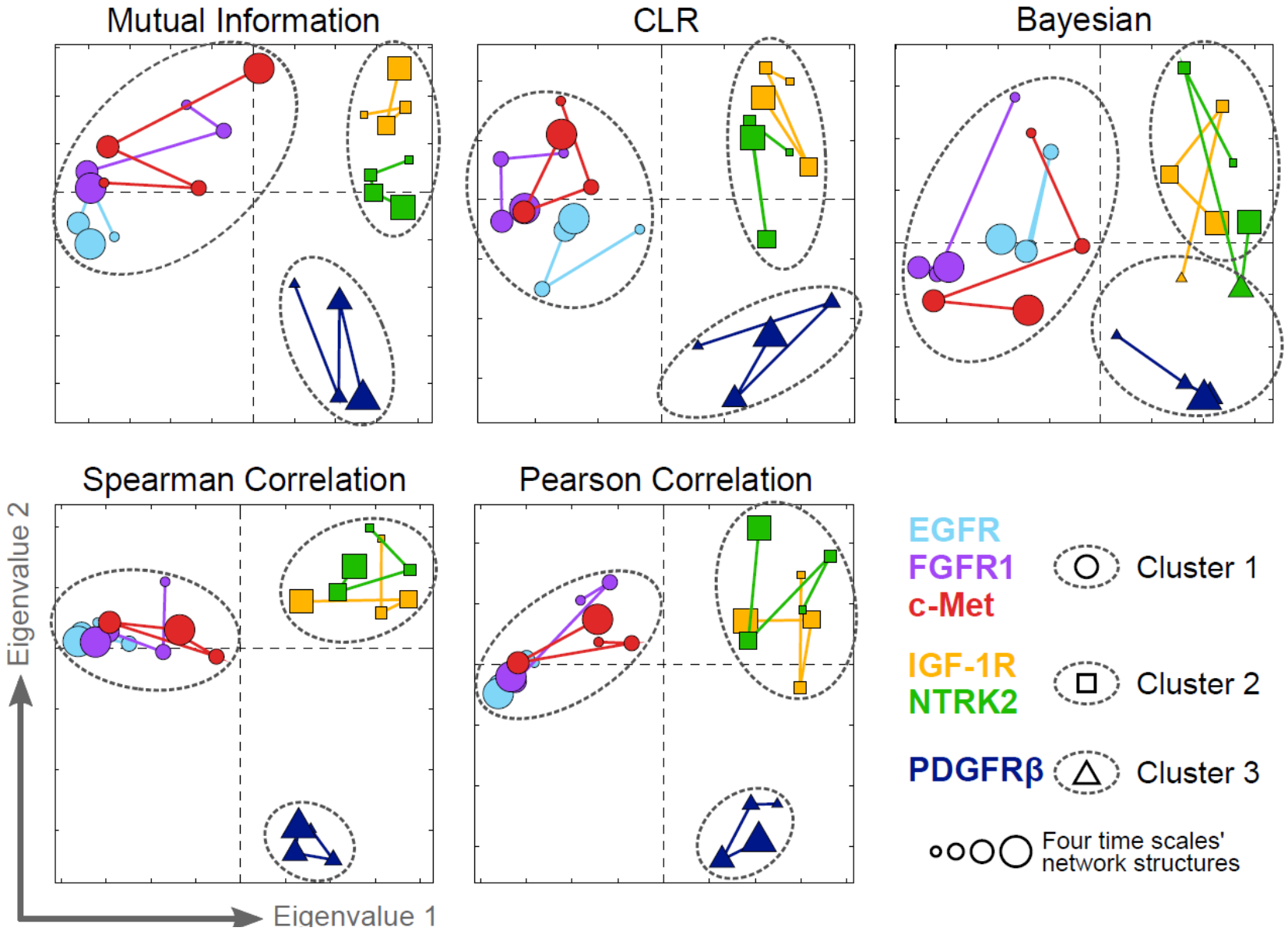
A consensus model for the 6 RTKs reveals a core signaling backbone and class specific interactions



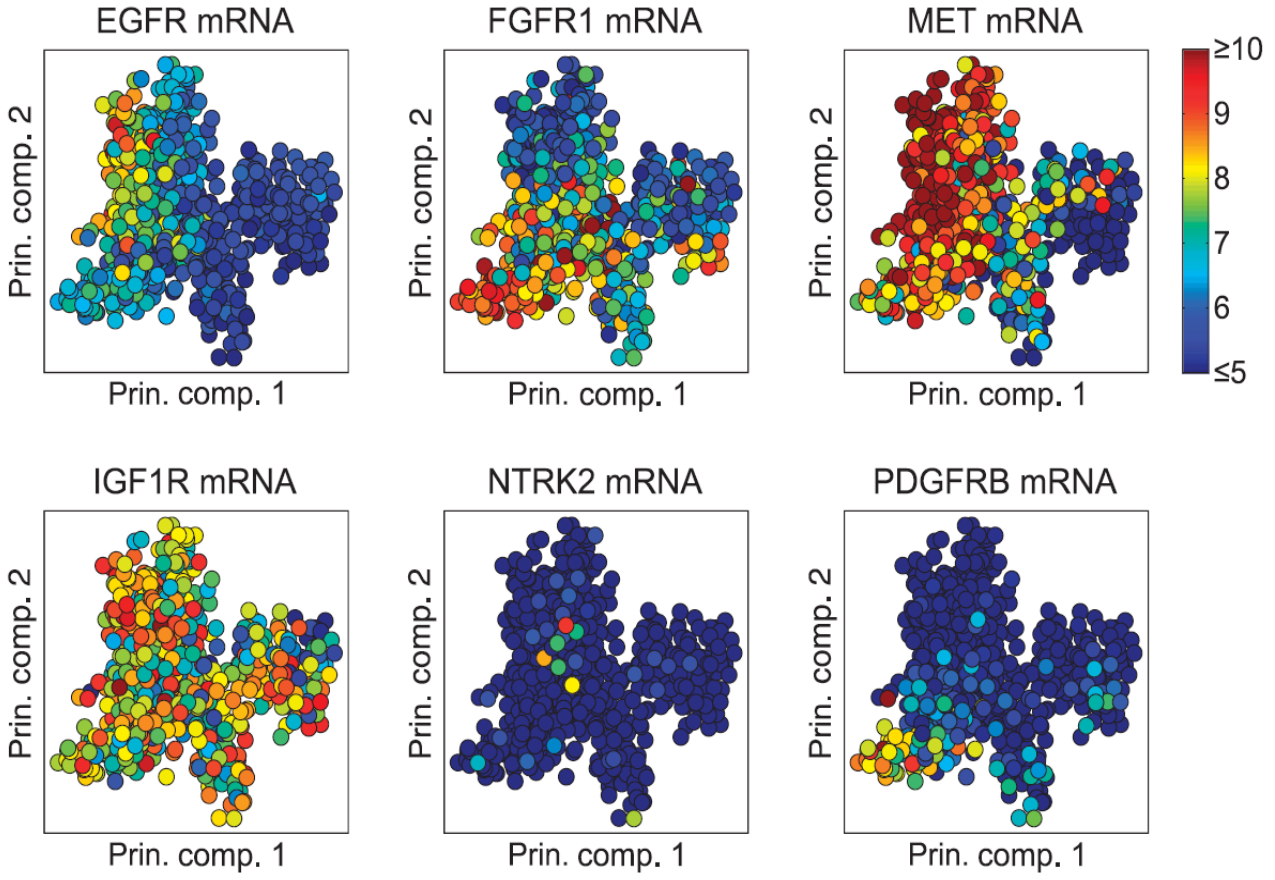


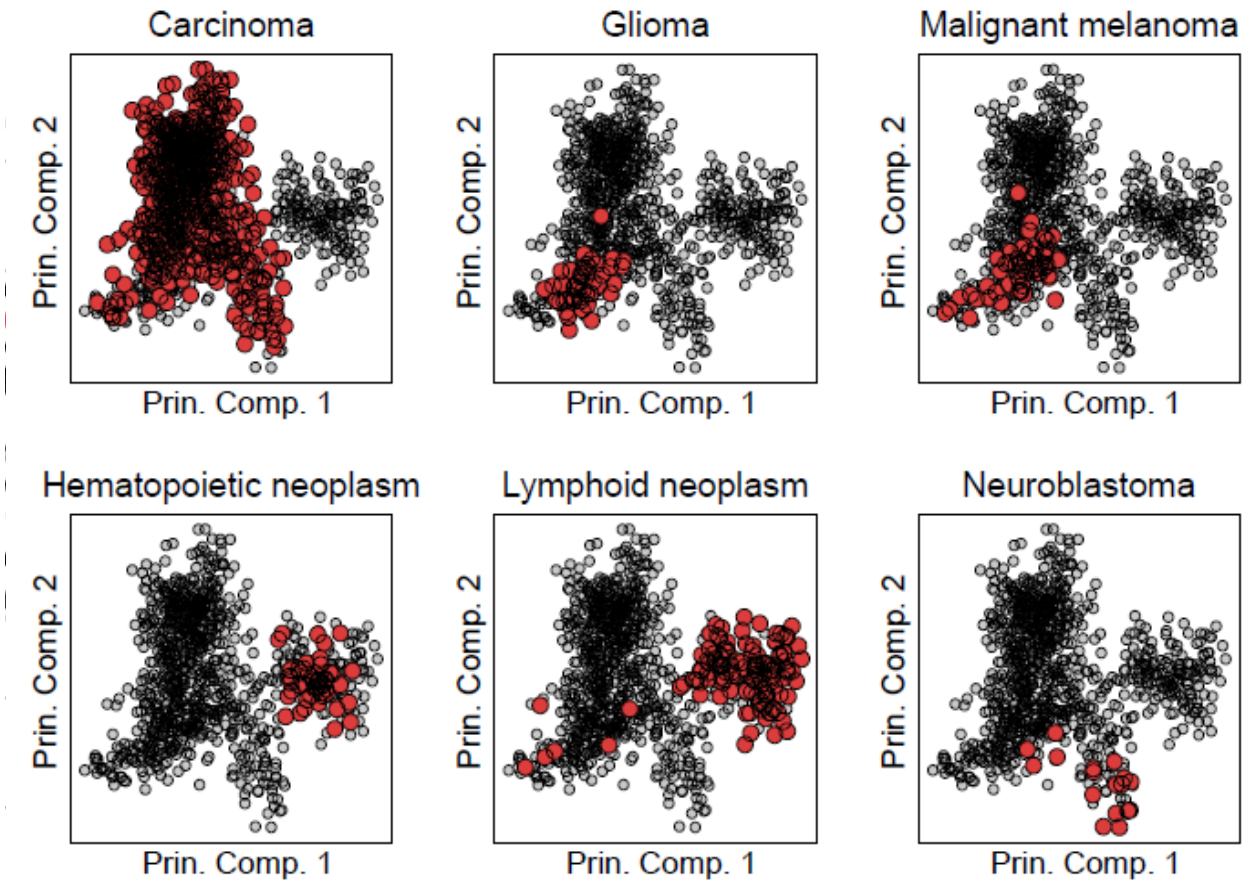


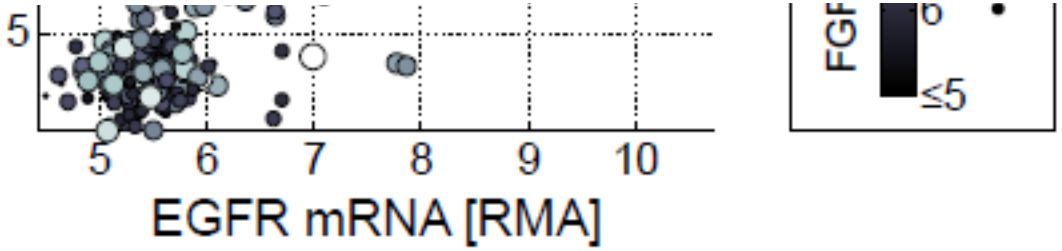
Clustering RTK-specific network models reveals 3 RTK classes.



EGFR, FGFR1 and MET co-expression in CCLE cell lines



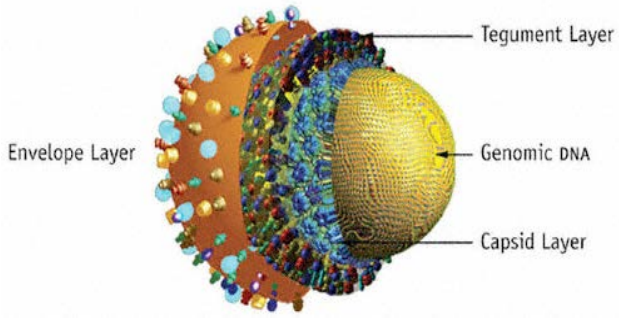




RTK expression correlates with decreased sensitivity to same-class RTK-directed drugs

Target/Receptor	EGFR	FGFR1	IGF1R	MET
EGFR (erlotinib)	1.26e-4	9.77e-6	0.15	0.15
FGFR1(TKI258)	4.38e-5	6.39e-3	7.25e-2	1.12e-3
IGF1R (AEW541)	0.105	0.541e-3	1.18e-5	2.72e-2
MET (PHA665752)	1.06e-4	0.14	0.36	0.13

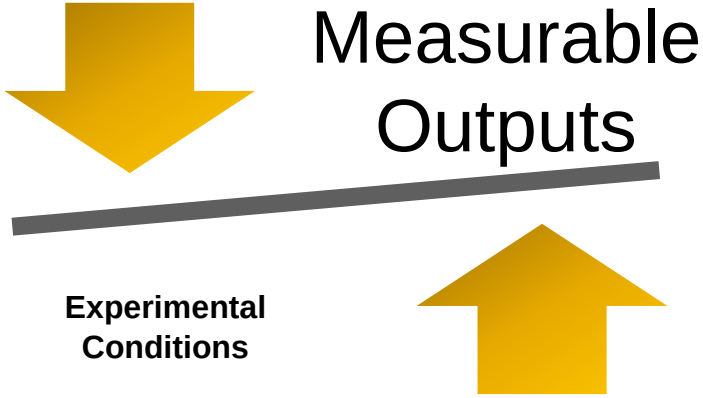
Case Study 2: Kaposi's Sarcoma-associated Herpesvirus (KSHV)



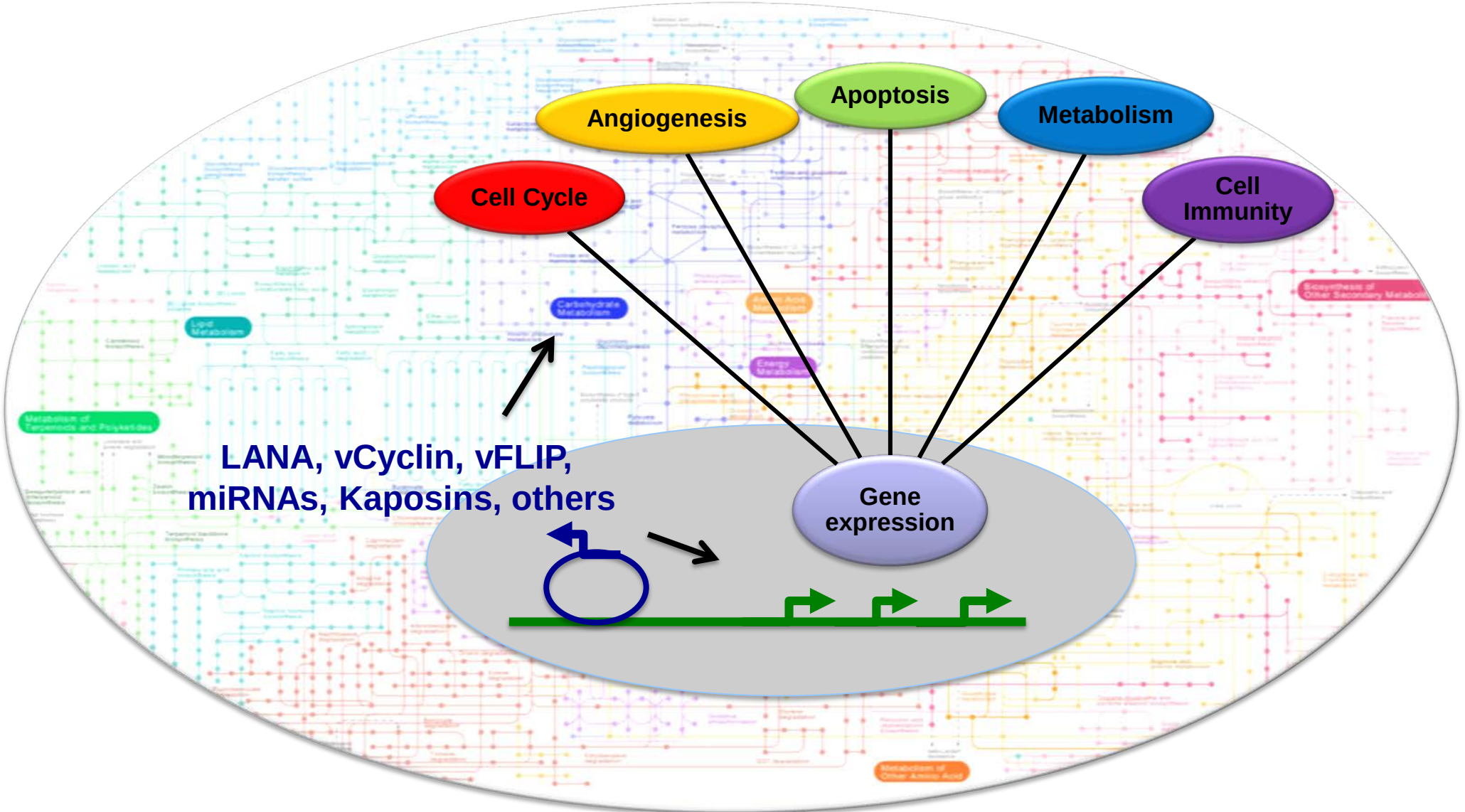
- Human gamma-herpesvirus

- Infectious agent of Kaposi's Sarcoma (KS)
  - Endothelial cell based tumor
  - Highly vascularized tumor

- KSHV presents both lytic and **latent** programs
  - KS tumors are predominantly latently infected
  - Only about 4 viral genes and miRNAs dominate this state



KSHV modulates cell signaling pathways to maintain a latent infection



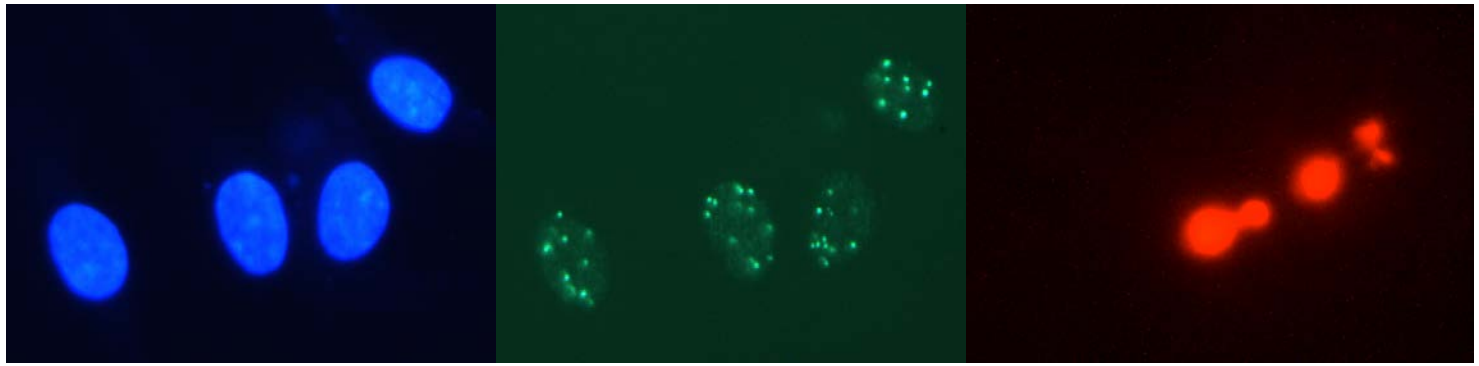
Endothelial cell culture system to study KSHV pathogenesis

- Tert- Immortalized Microvascular Endothelial cells (TIME)
- Human Dermal Microvascular Endothelial Cells (1º hDMVECs)
- Establishment of latency occurs at 48 hours post infection (hpi)
- KSHV infection rates are detected by Immunofluorescence
  - >90% latent, <1-5% lytic (similar to KS tumors)

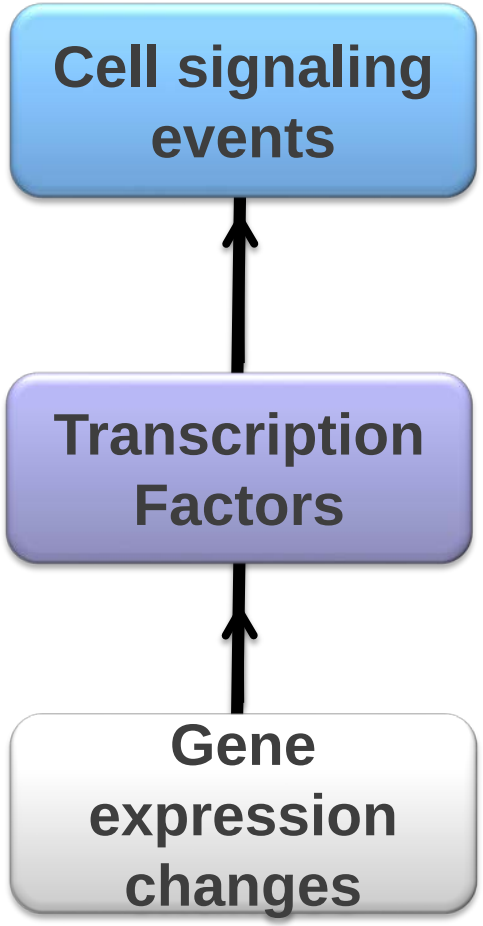
Blue: DAPI
(cell nuclei)

Green: LANA
(Latent protein marker)

Red: ORF59
(Lytic protein marker)



Applying high-throughput technologies to unravel complex signaling networks

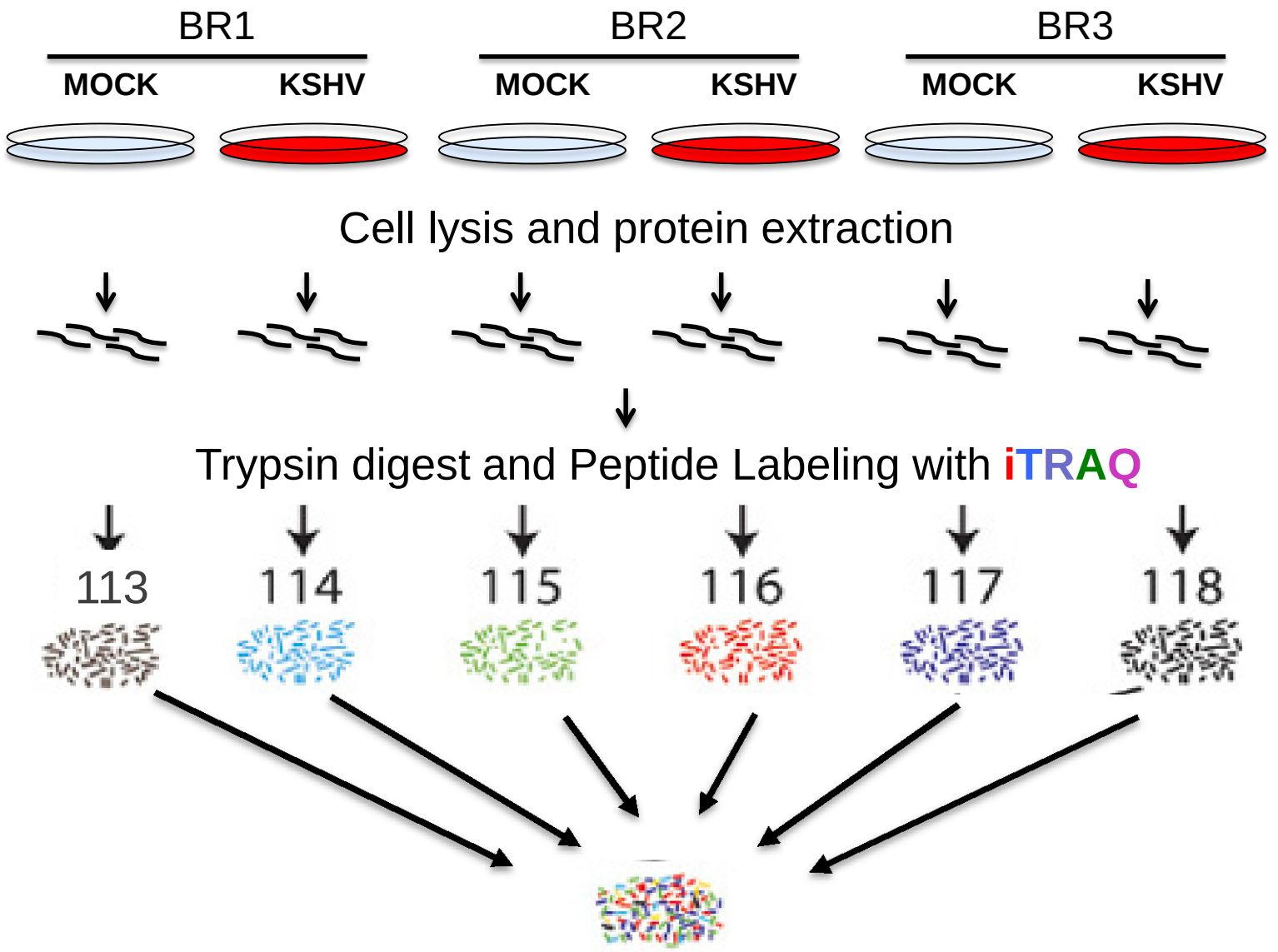


- Quantitative mass spectrometry-based:
  - Proteomics
  - Phosphoproteomics

- Link upstream signaling with gene expression using network inference
  - Steiner Forest algorithm

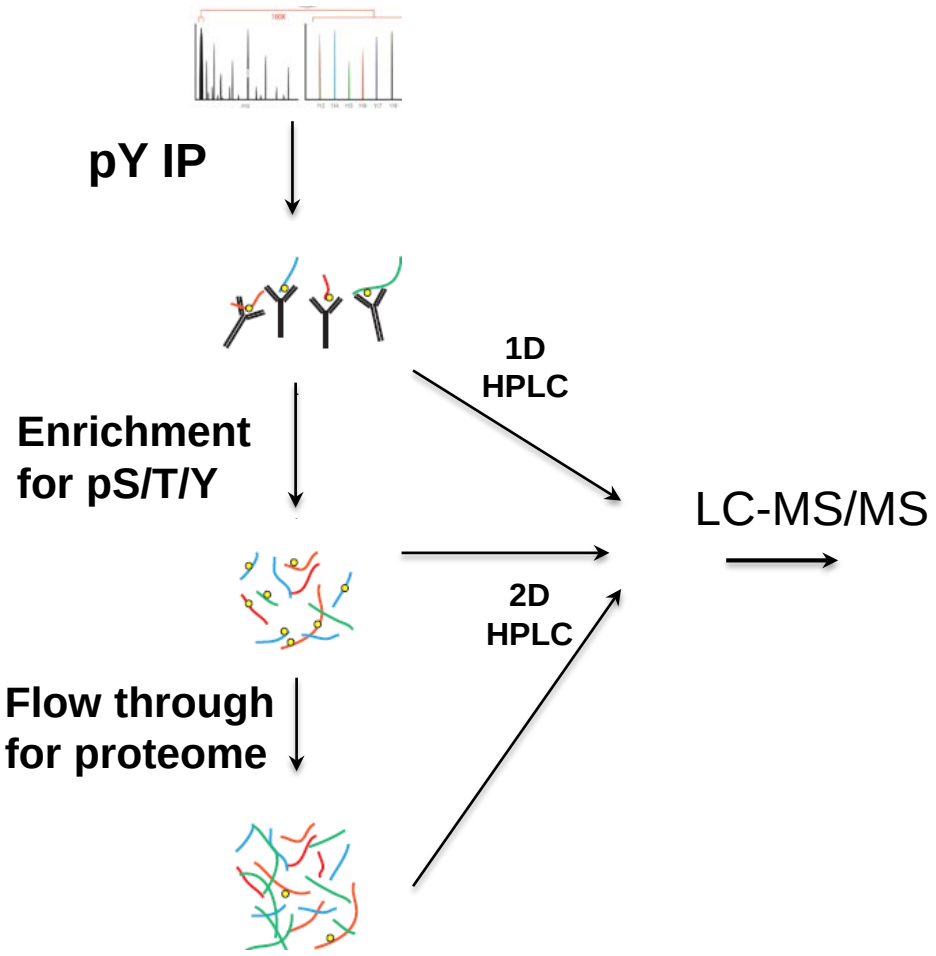
- Transcriptomics to evaluate differential gene expression
  - Infer Transcription Factor activation changes

Proteomics workflow using TIME cells

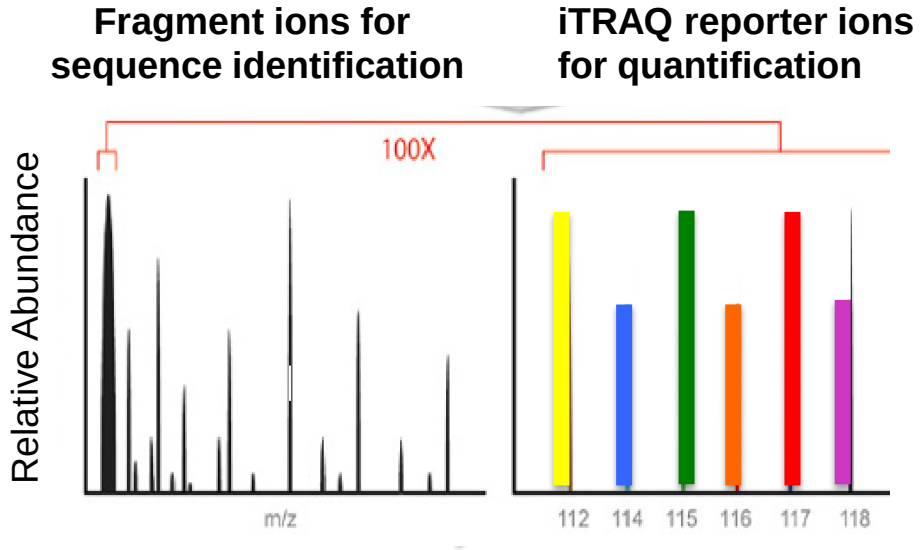


BR= Biological Replicate

Relative peptide quantification using iTRAQ



LC-MS/MS



Data Analysis

3 Biological replicate (samples)
2 Technical replicates (runs)

A small subset of (phospho)-proteins changes in response to KSHV infection

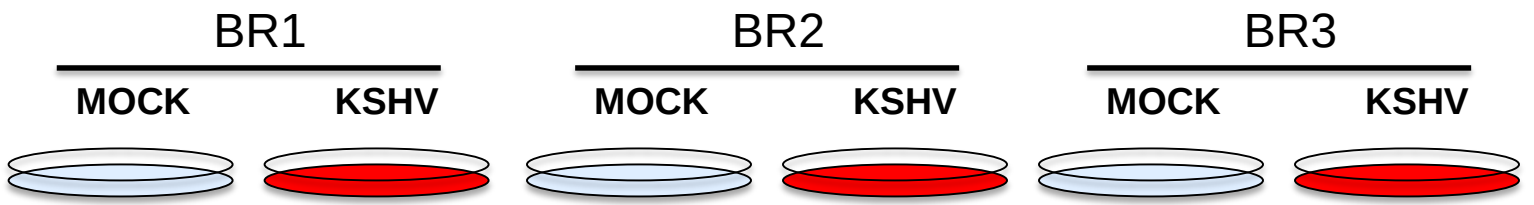
Proteome

SEQUEST FDR 1%		Total # proteins	# of proteins altered
		5304	
p<.01	Upregulated	164	
	Downregulated	125	

Phospho-Proteome

SEQUEST FDR 1%		Total # Unique pProteins	Total # Unique pPeptides
		2963	1469
p<.01	Upregulated	88	
	Downregulated	87	

Transcriptomics workflow using TIME cells



48HPI

Cell lysis and **RNA** extraction

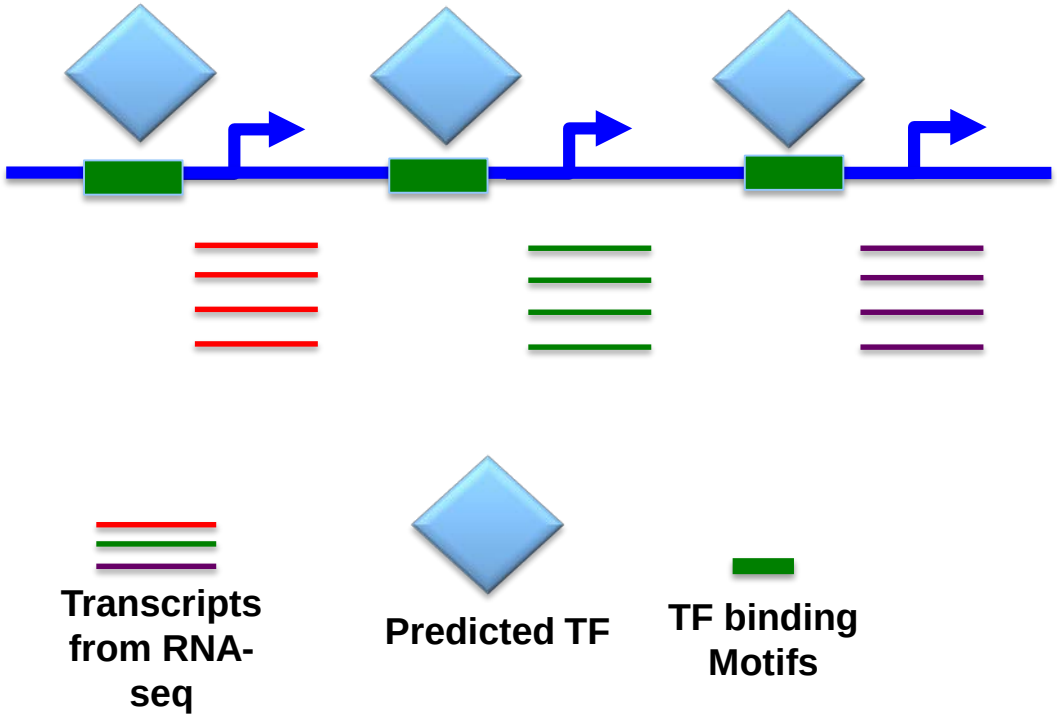
RNA to cDNA

Illumina next-generation sequencing

List > 11,000 genes

BR= Biological Replicate

Using gene expression profiles to predict possible transcription factor binding sites



1. Identify the significantly altered transcripts

1. Rank TF binding motif occurrence
   - 446 motifs
   - 1 Kb from TSS

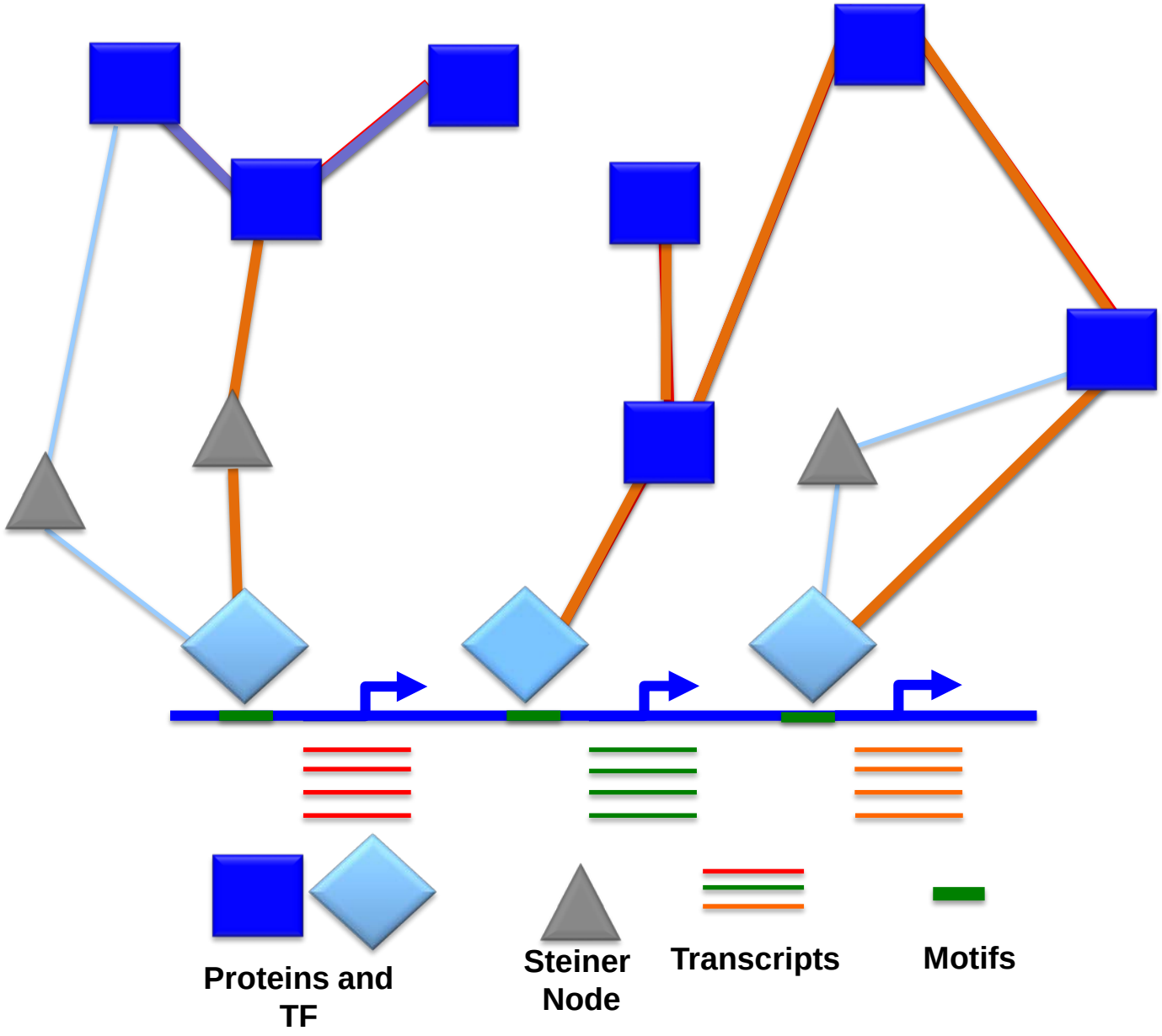
2. Infer active and repressing transcription factor (TF)

Many motifs show significant enrichment of up-regulated flanking transcripts

- Use Wilcoxon Rank sum test to identify cis-regulatory motif enrichment
- 236 TF binding motifs change between mock and KHSV infection (5% FDR)
- 55% of the TF are predicted to be activating

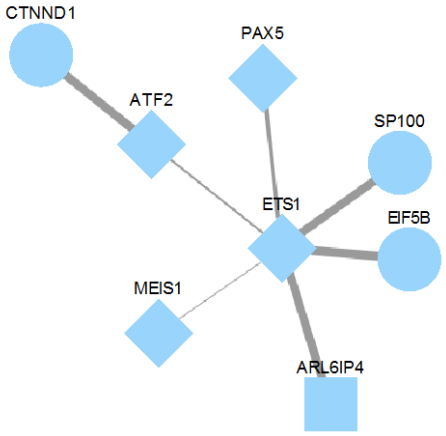
TF	two-tailed pvalue	FDR
S8	5.38E-11	2.39E-08
SRY	3.59E-10	8.00E-08
RSRFC4	8.28E-10	1.23E-07
FOXJ2	3.60E-09	4.00E-07
OCT1	5.32E-09	4.05E-07
LHX3	5.46E-09	4.05E-07
POU6F1	1.01E-08	5.72E-07
MEIS1	1.03E-08	5.72E-07
BACH2	1.24E-08	6.08E-07
HFH1	1.50E-08	6.08E-07
MEF2	1.87E-08	6.92E-07
NKX25	3.94E-08	1.35E-06
GTF2I	5.99E-08	1.90E-06
NRF2	4.00E-03	9.58E-03
ETS1	2.38E-02	4.38E-02
STAT3	3.17E-02	5.76E-02

Integration of Proteomics/Transcriptomic Data Using Steiner Tree

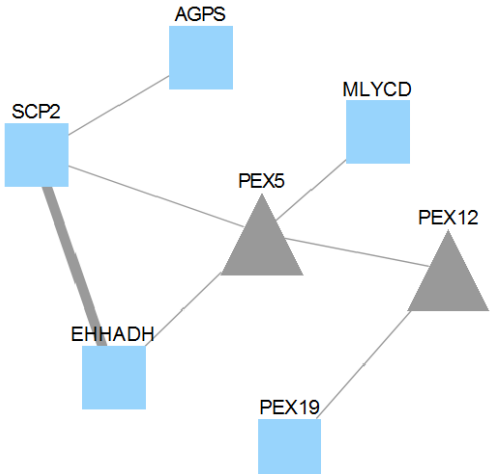


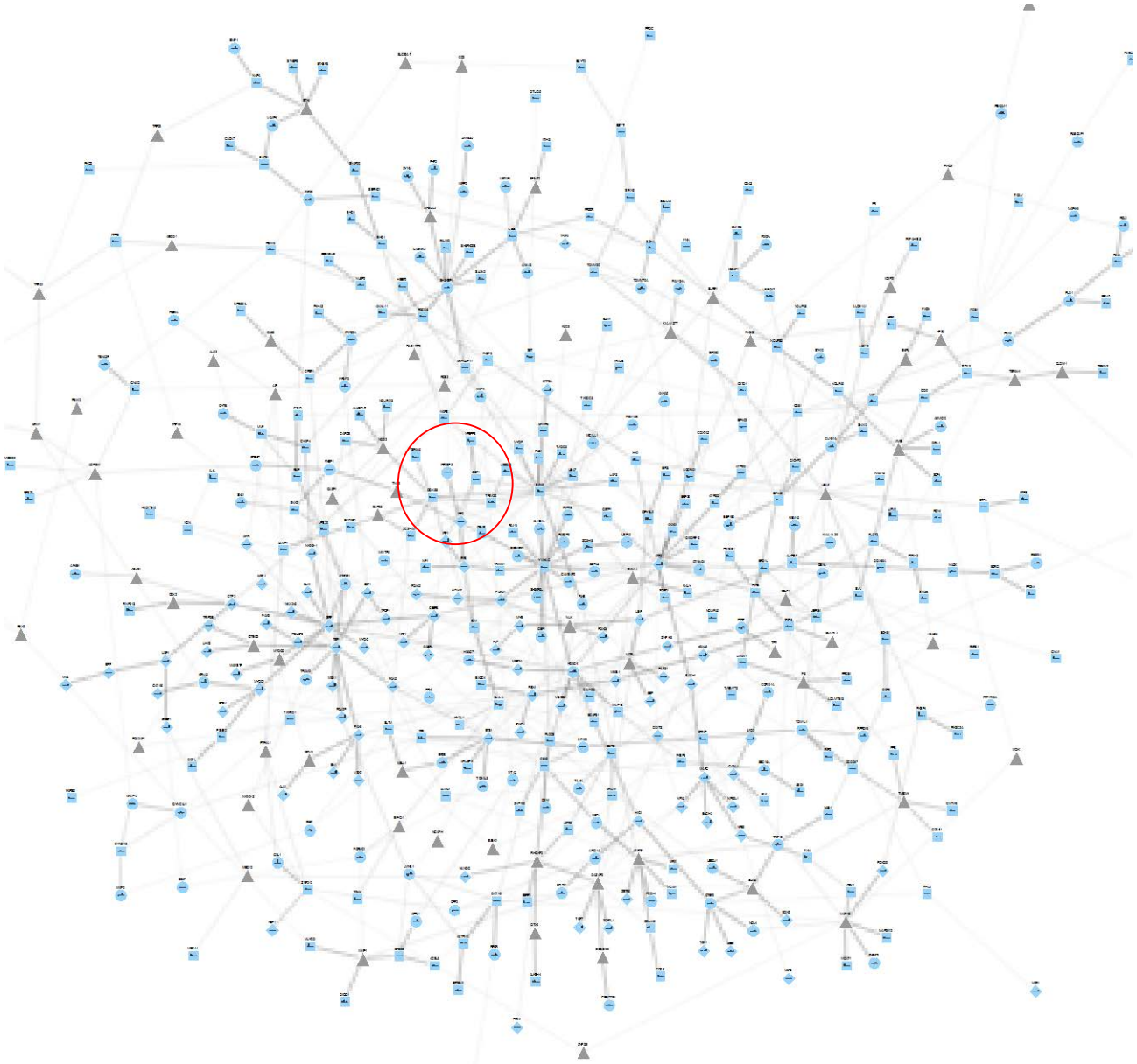
Network of KSHV infected endothelial cells

Pluripotency of stem cells and MAPK Signaling

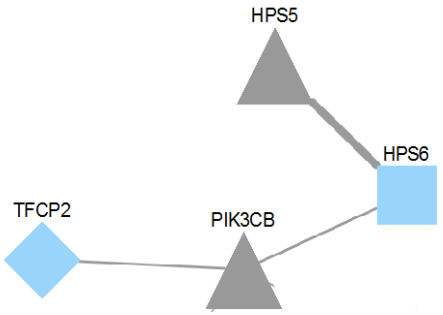


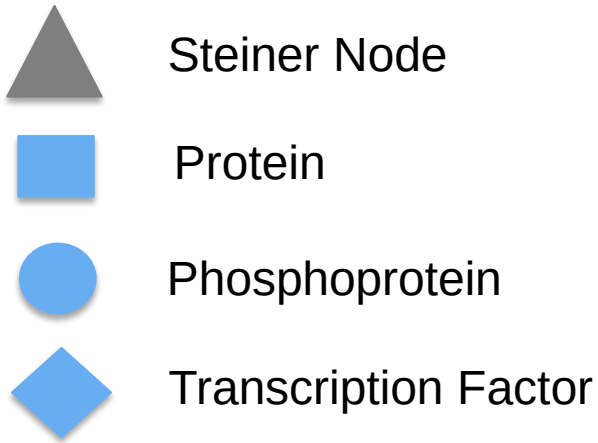
Peroxisome Biogenesis



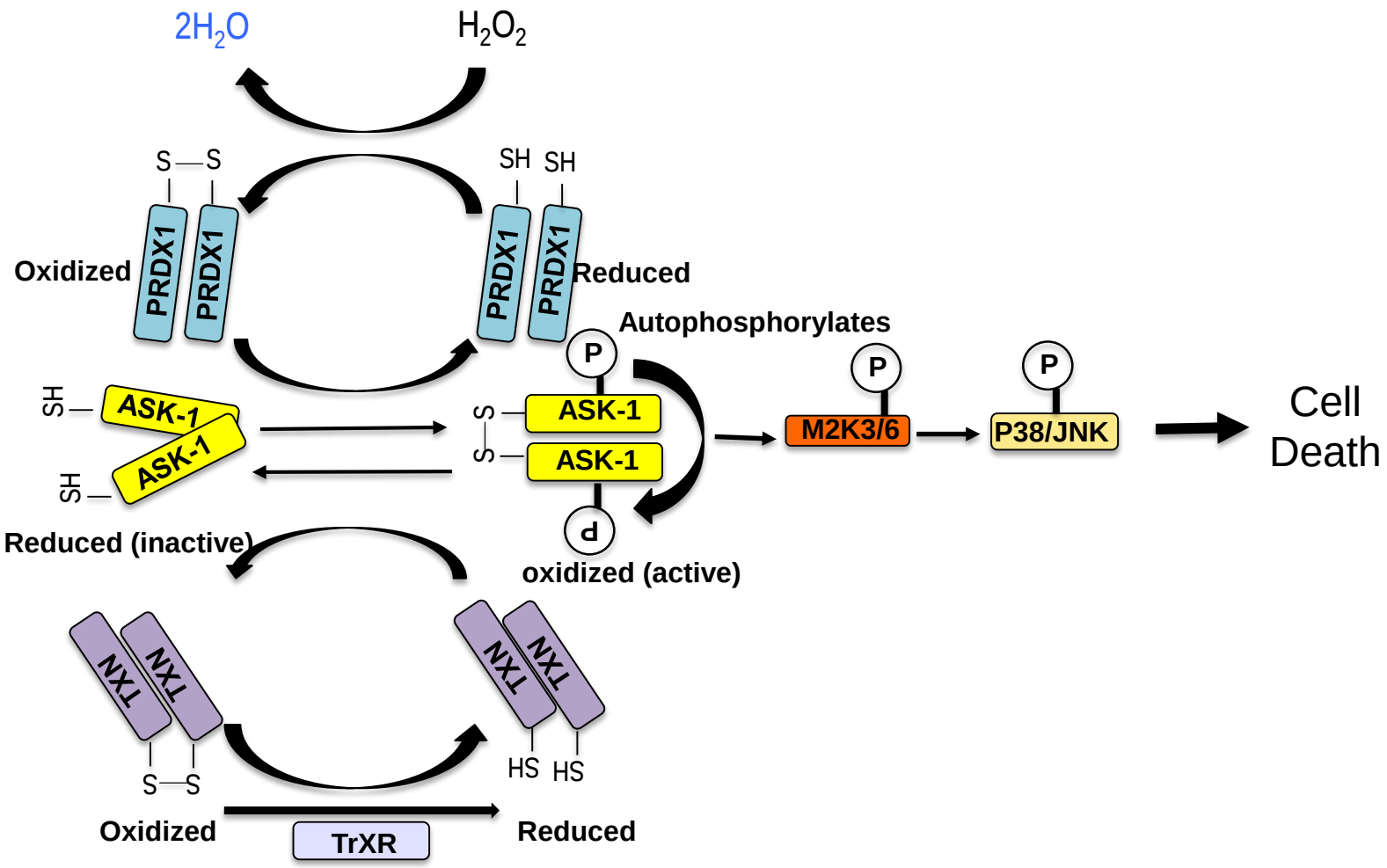


mTOR and Lysosome synthesis





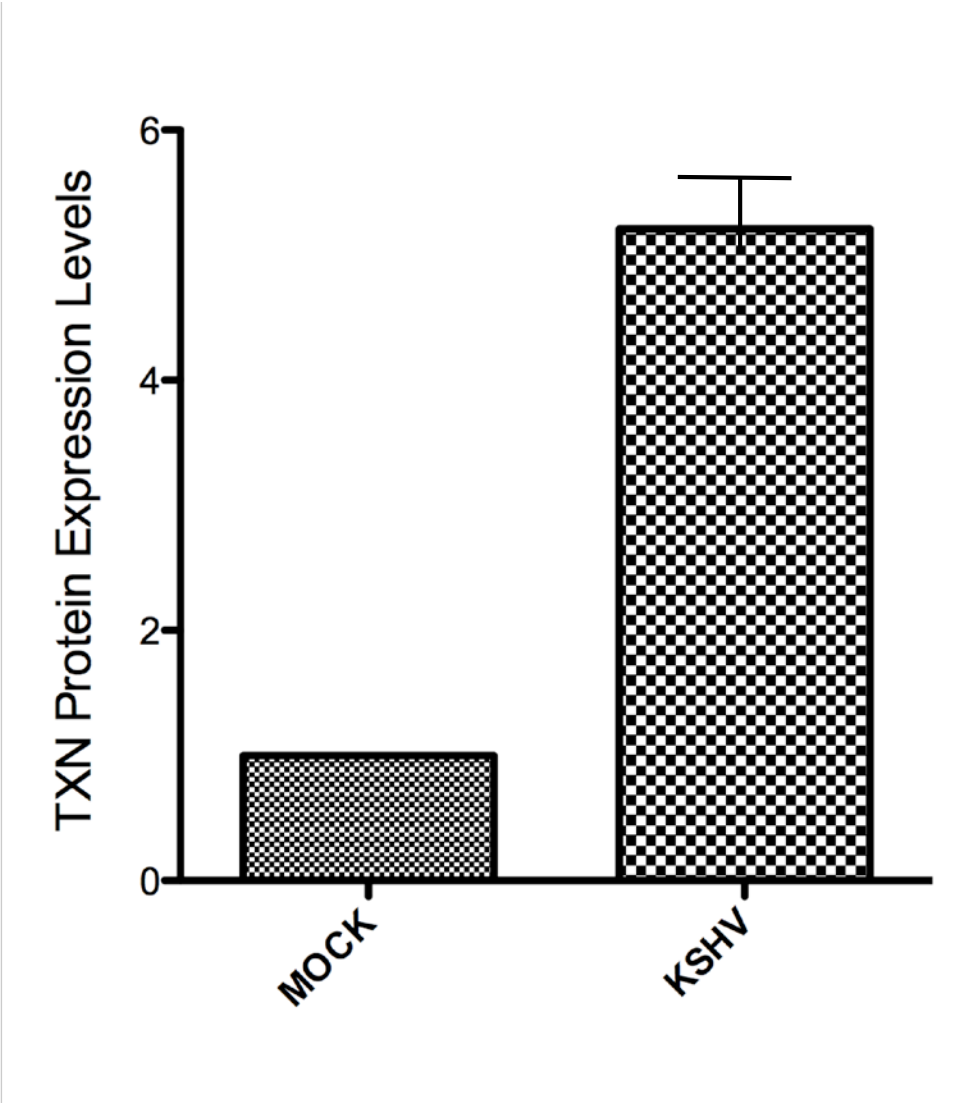
The Thioredoxin System

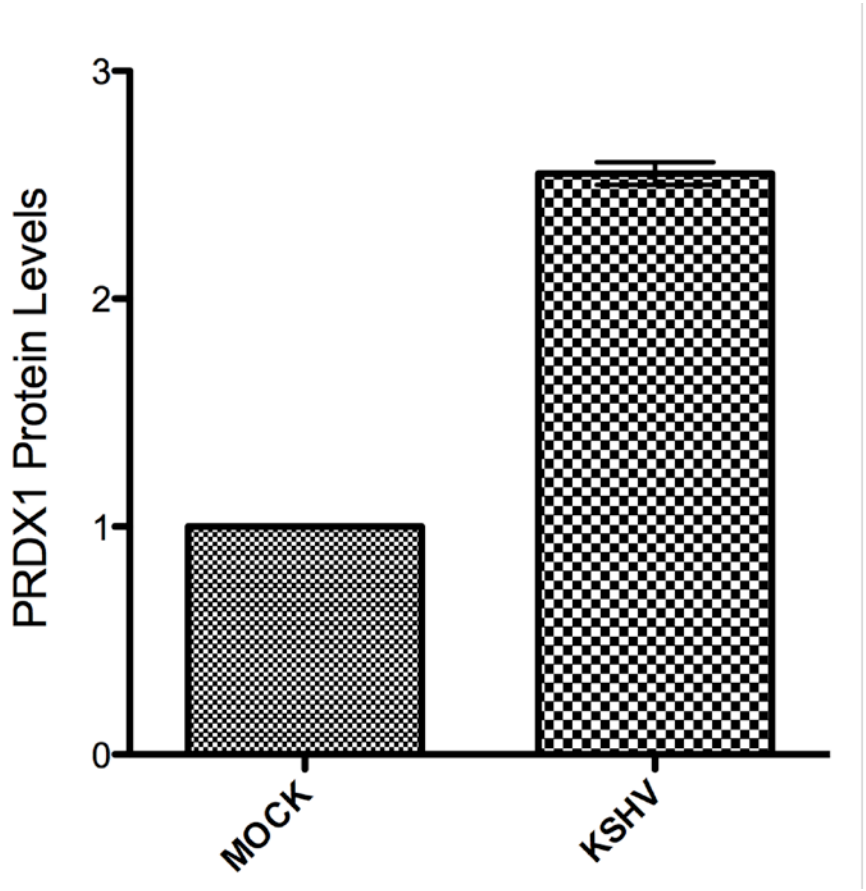


Ledgerwood et. al. Free Radical Biology and Medicine Oct. 2012

Latent KSHV infection modulates the PRDX/TXN system to regulate hydrogen peroxide and to prevent endothelial cell death

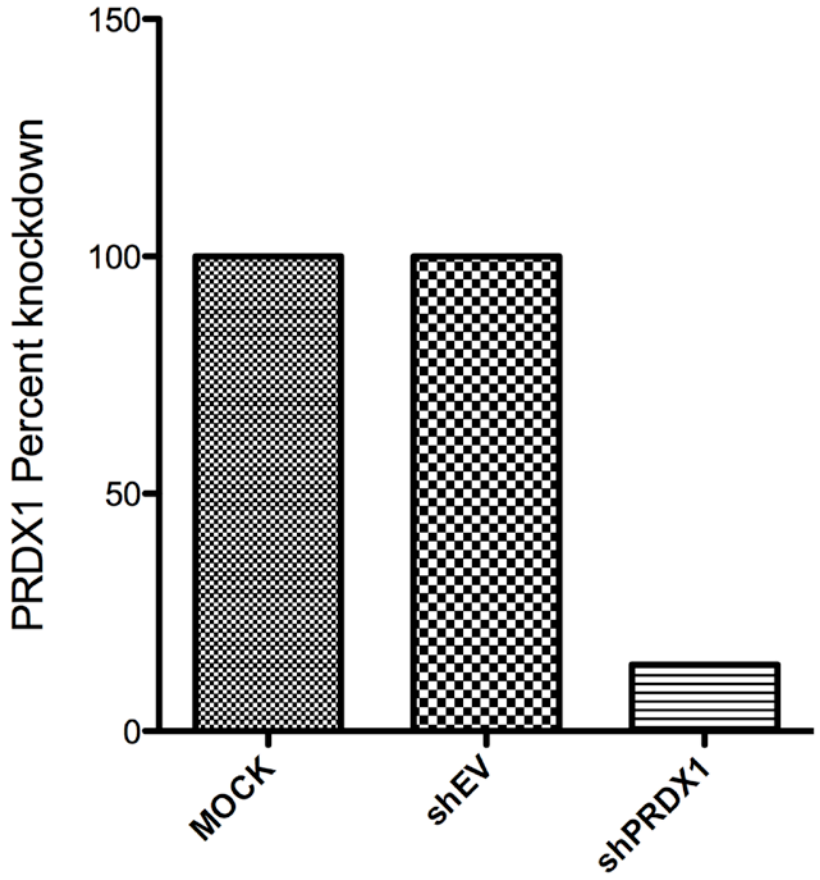
PRDX1and TXN levels are increased in KSHV infected endothelial cells



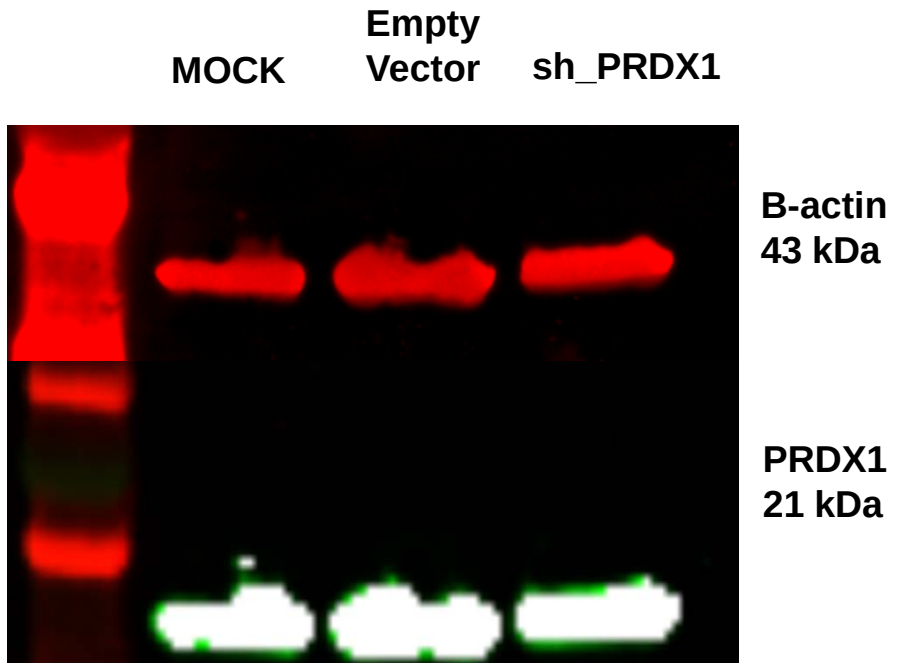


PRDX1 gene and protein expression levels are efficiently silenced

Gene Expression

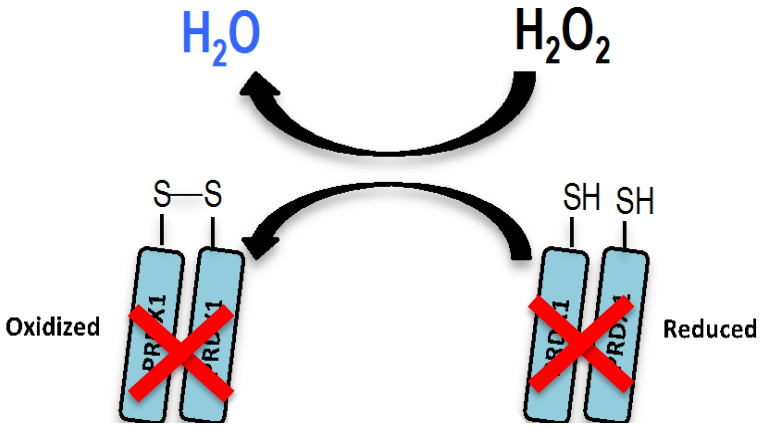


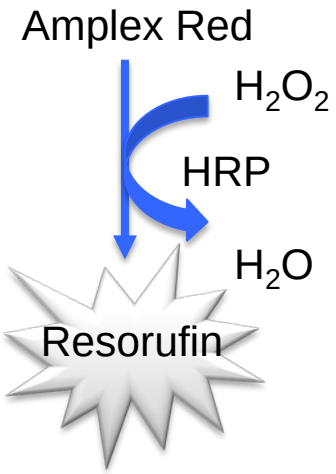
Protein levels

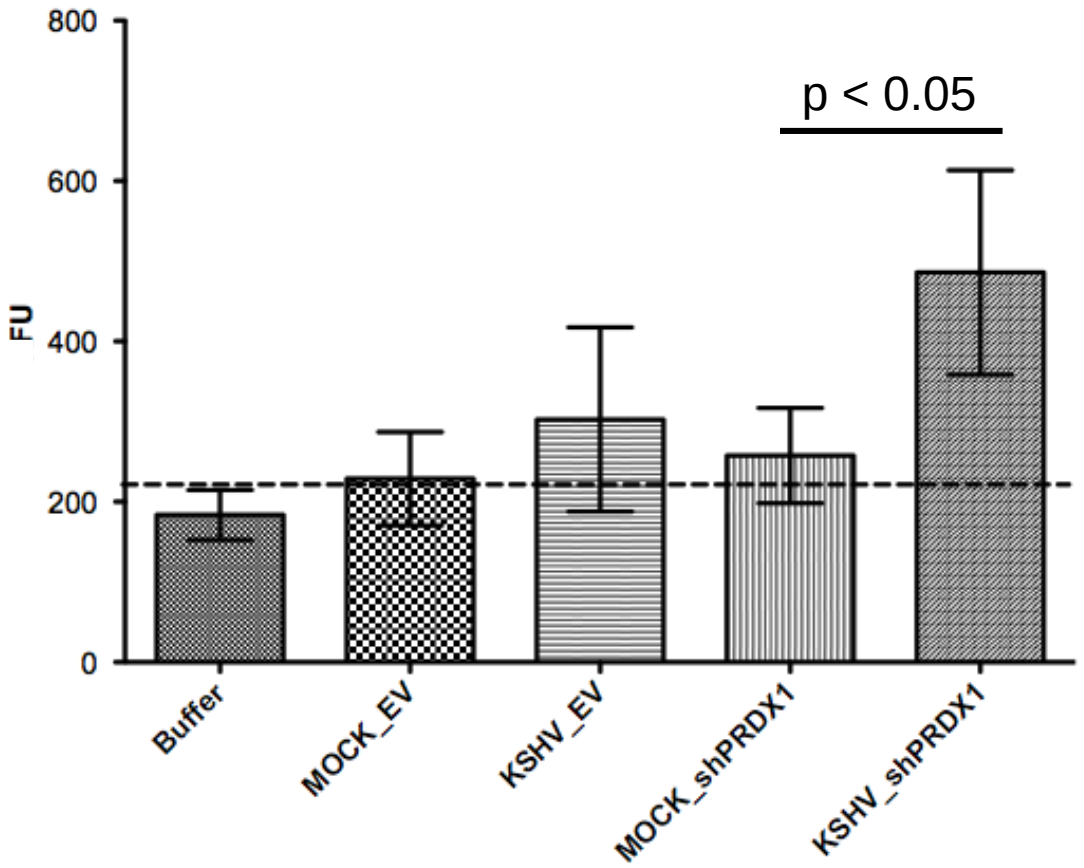


Data was normalized against GAPDH and HPRT

H_2O_2 Levels are increased in the absence of PRDX1 during KSHV infection

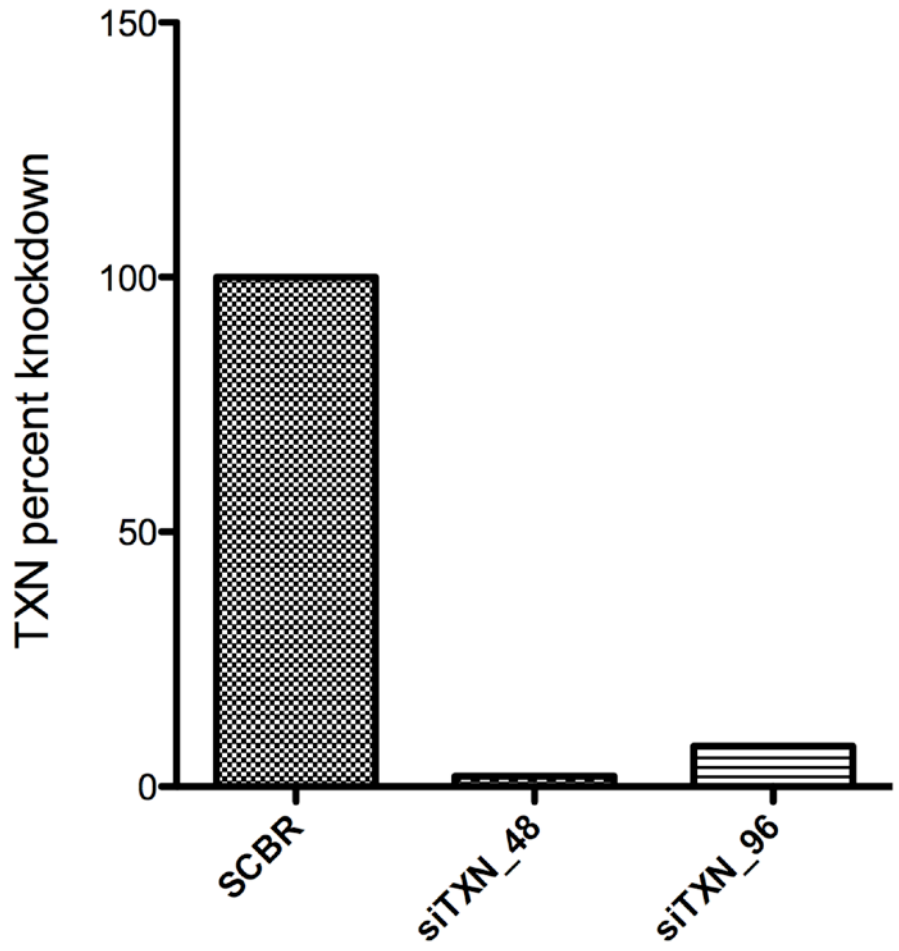






Normalized against total number of cells

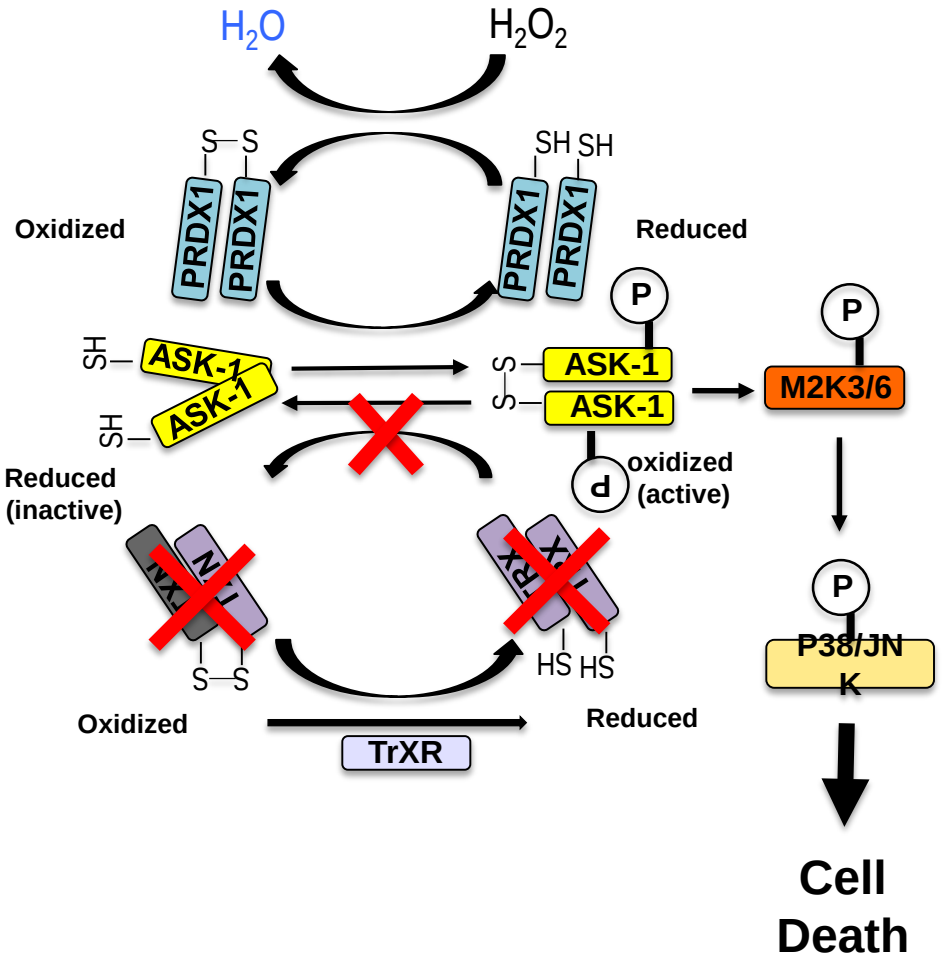
TXN gene expression is efficiently silenced

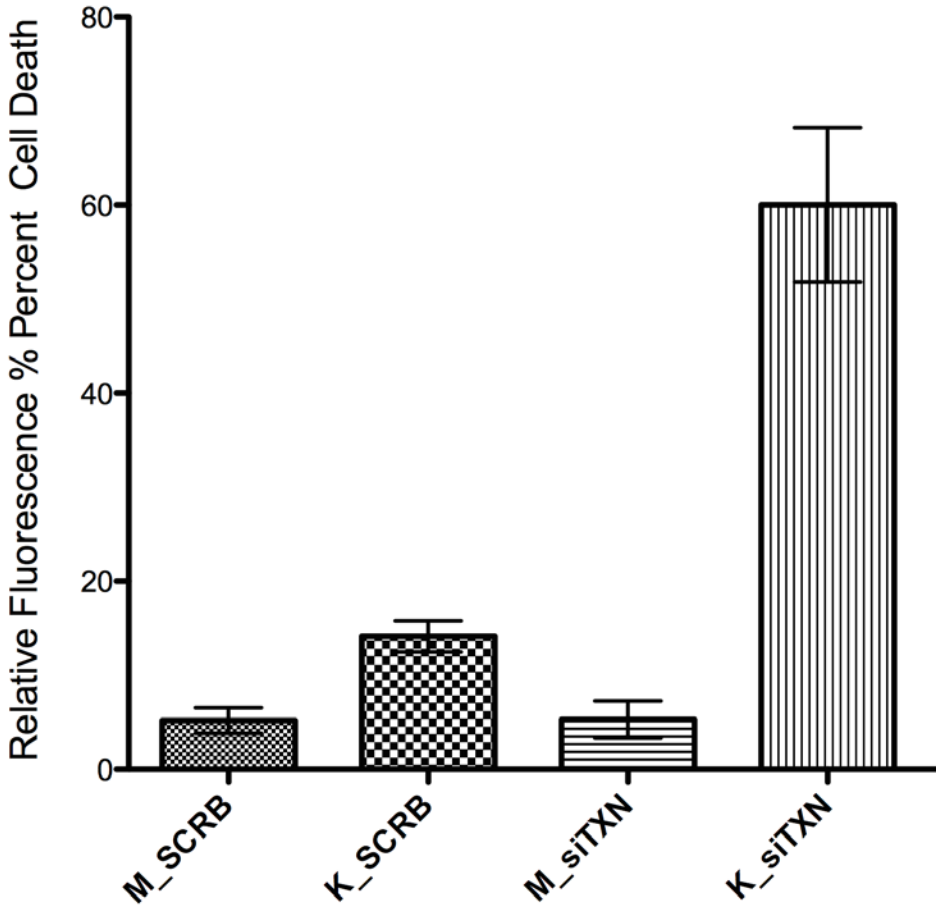


qRT-PCR

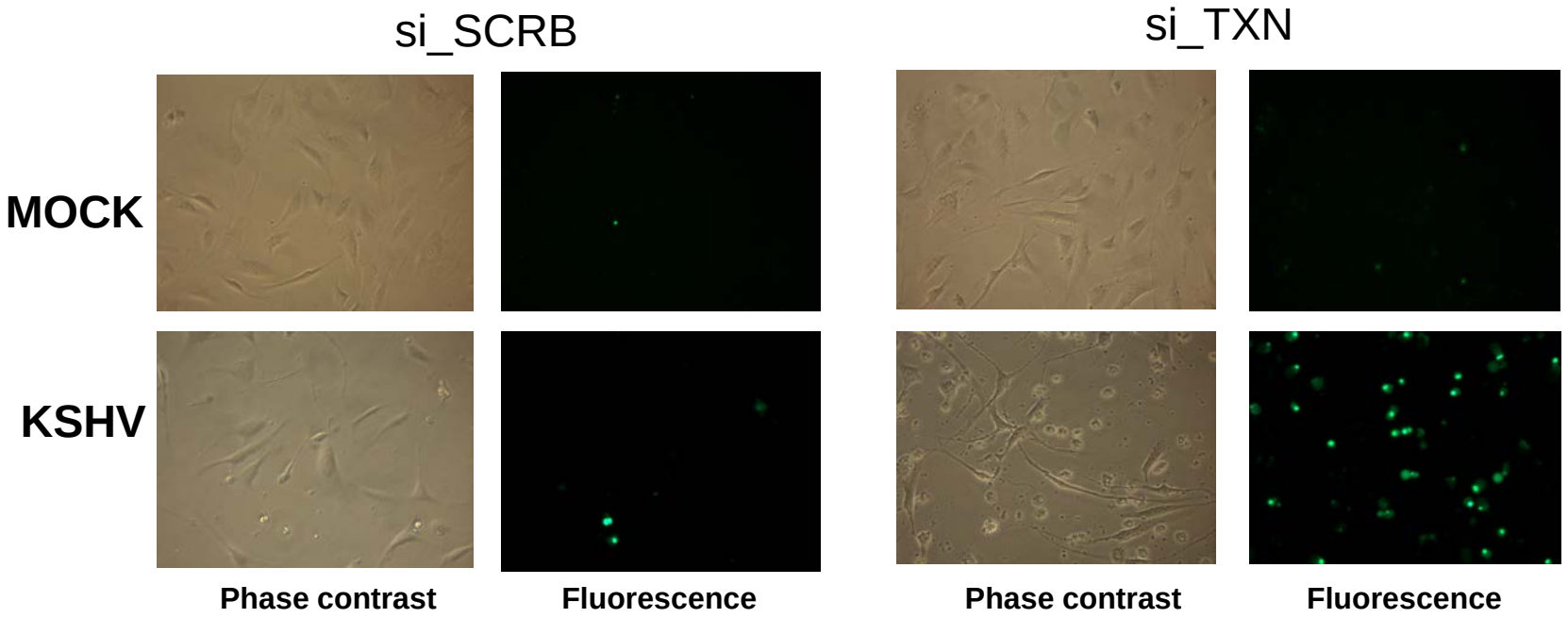
Data was normalized against GAPDH and HPRT

TXN knockdown during latent KSHV infection leads to increase cell death

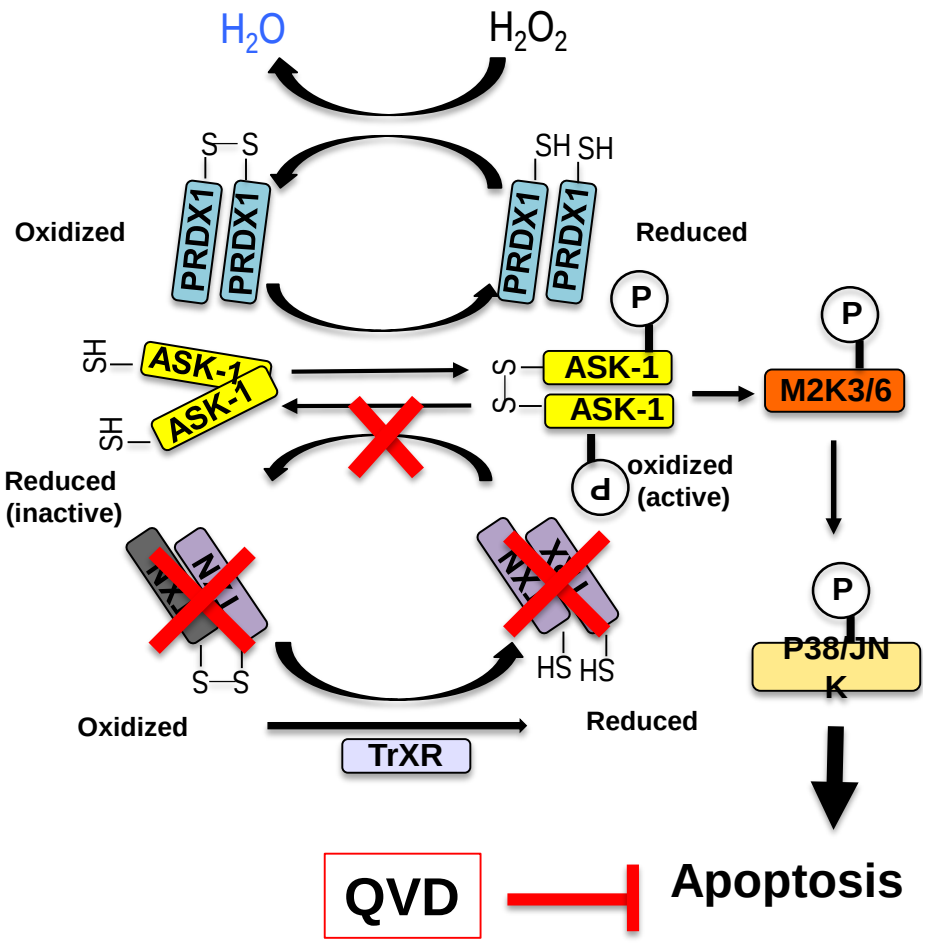


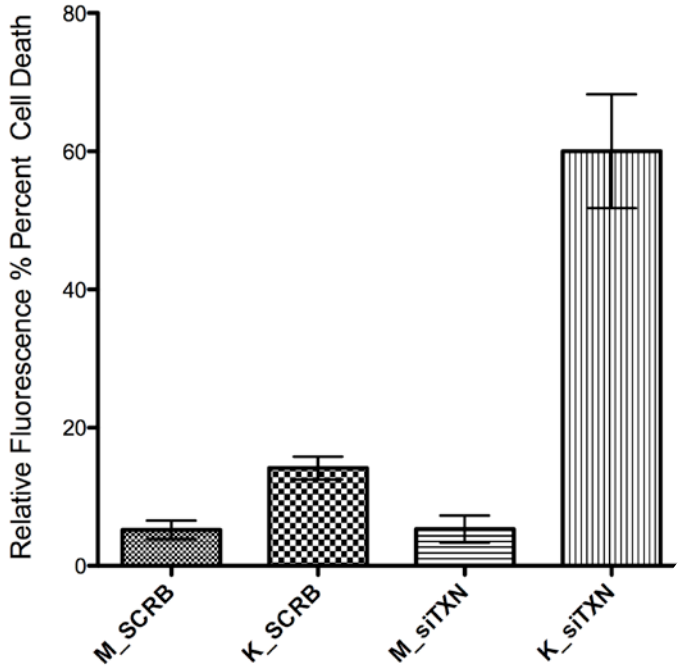


TXN knockdown during latent KSHV infection leads to increase cell death

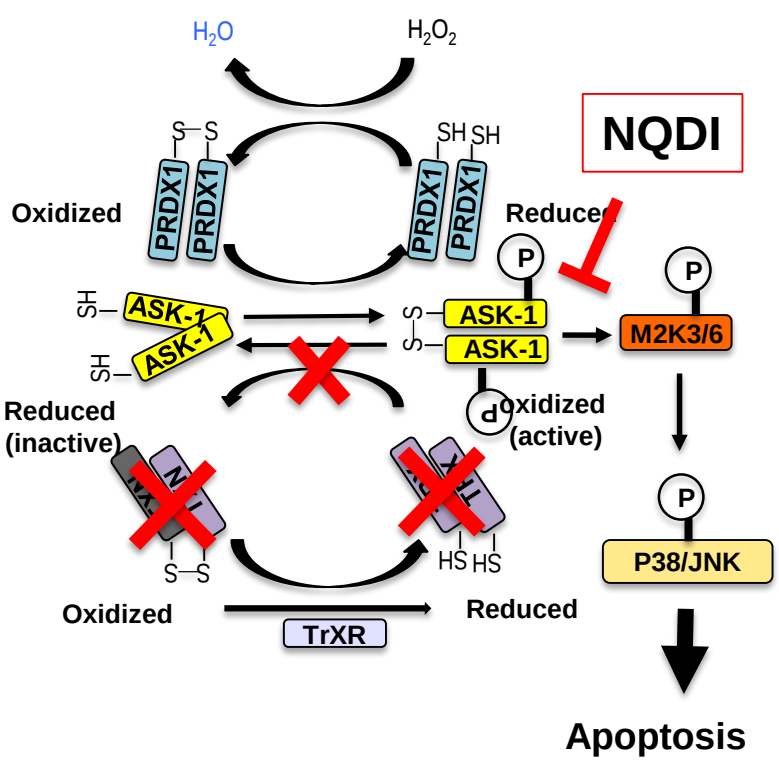


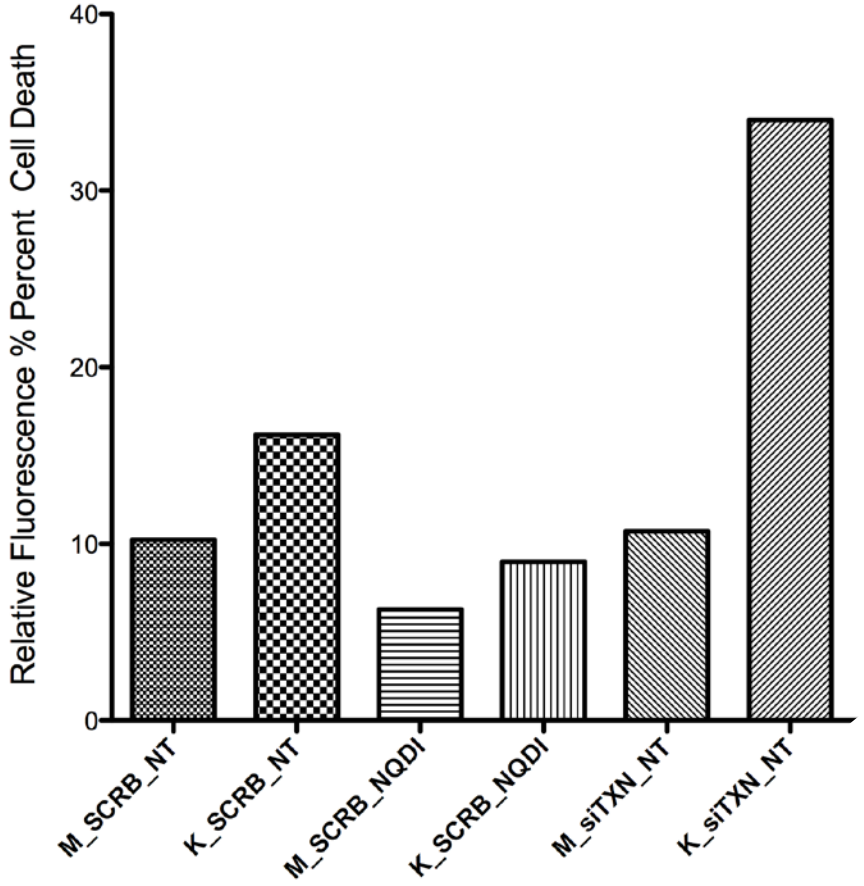
Cell death seems to occur through apoptosis





Cell death seems to be a function of ASK activity





Acknowledgements

RTK Project

Gavin Macbeath
Doug Lauffenburger
Mark Sevecka
Joel Wagner

KSHV Project

Nathan Camp
Anthony Gitter
Alex Hu
Zoi Villasana