A Two-Dimensional ERK-AKT Signaling Code for an NGF-Triggered Cell-Fate Decision

Journal Club, January 2012

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Jia-Yun Chen, Jia-Ren Lin, Karlene A. Cimprich, Tobias Meyer, *Molecular Cell* 2012, 45(2):196-209.

Noise in cells - Part III

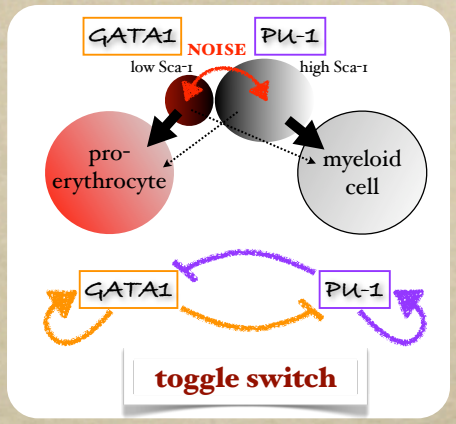
The premise:

Cells with identical

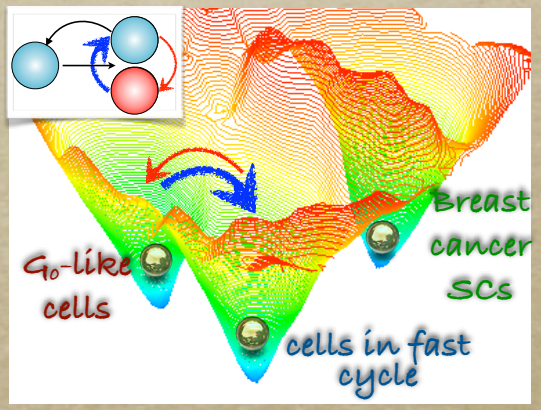
- genome
- phenotype
- environment
- history of environments

can display functionally heterogeneous behavior

2010



2012



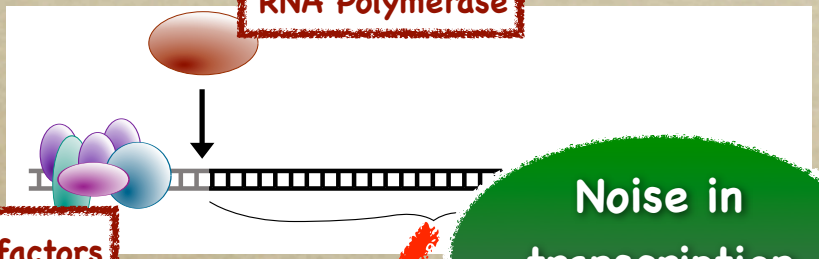
2013

Today: we look at signaling

Sources of noise in eukaryotic cells

Noise in TF availability

RNA Polymerase



Transcription factors

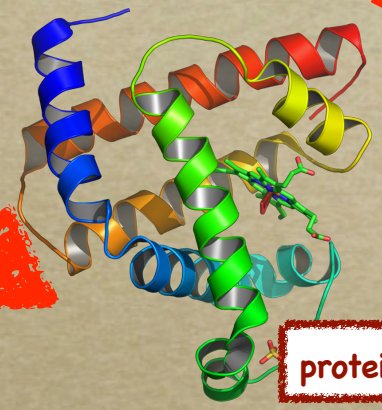
Noise in transcription initiation

Correlated fluctuation within pathways

Noise reduction/ amplification

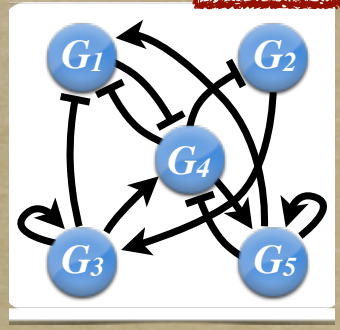
mRMA

Noise in translation



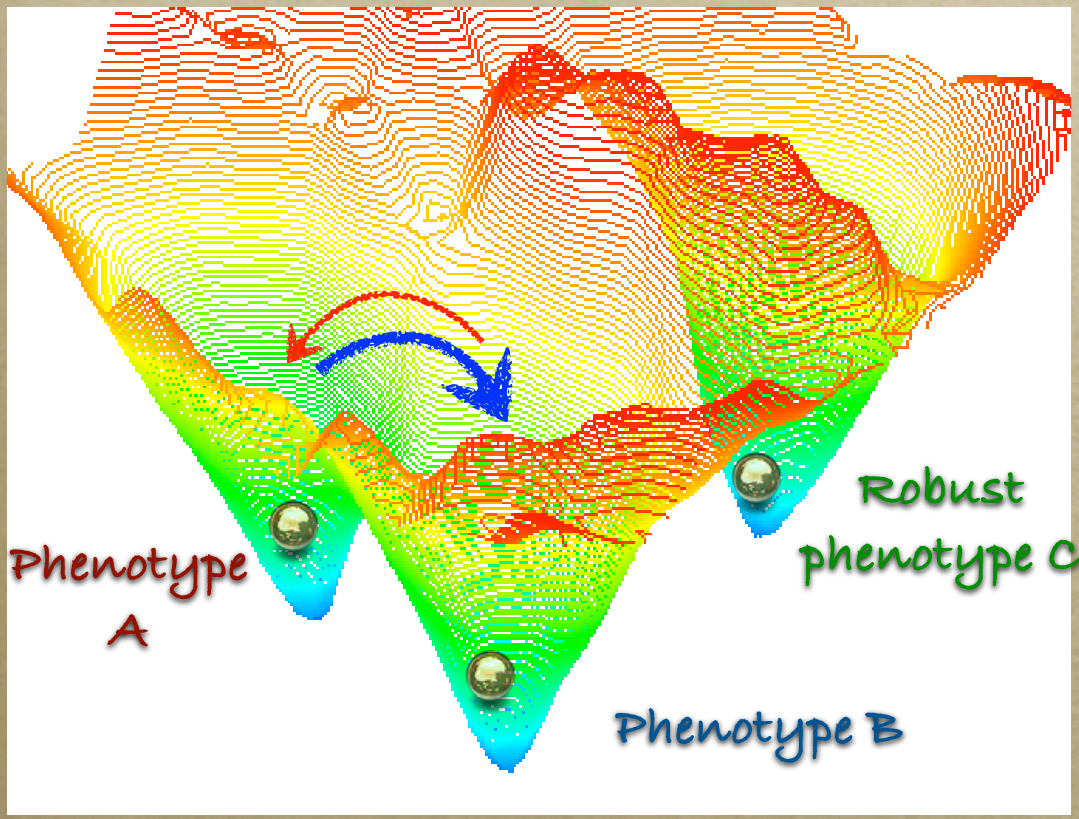
protein

regulatory circuit



~30% variability in protein expression

Consequences of noise in eukaryotic cells



- Spontaneous phenotypic heterogeneity

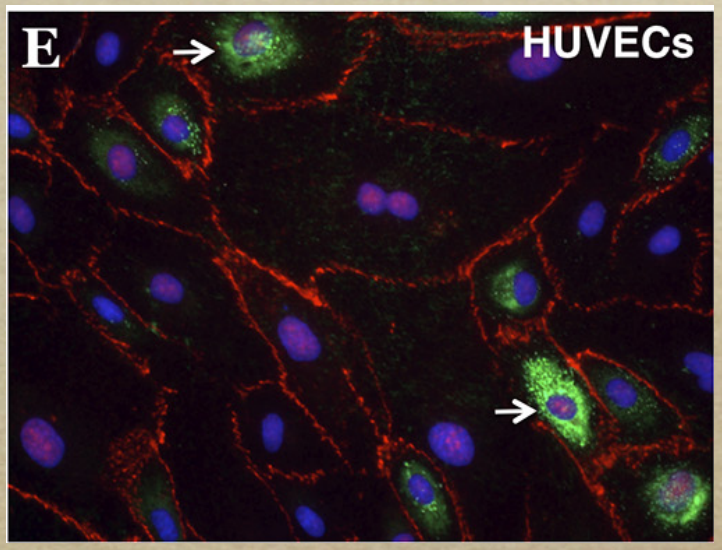
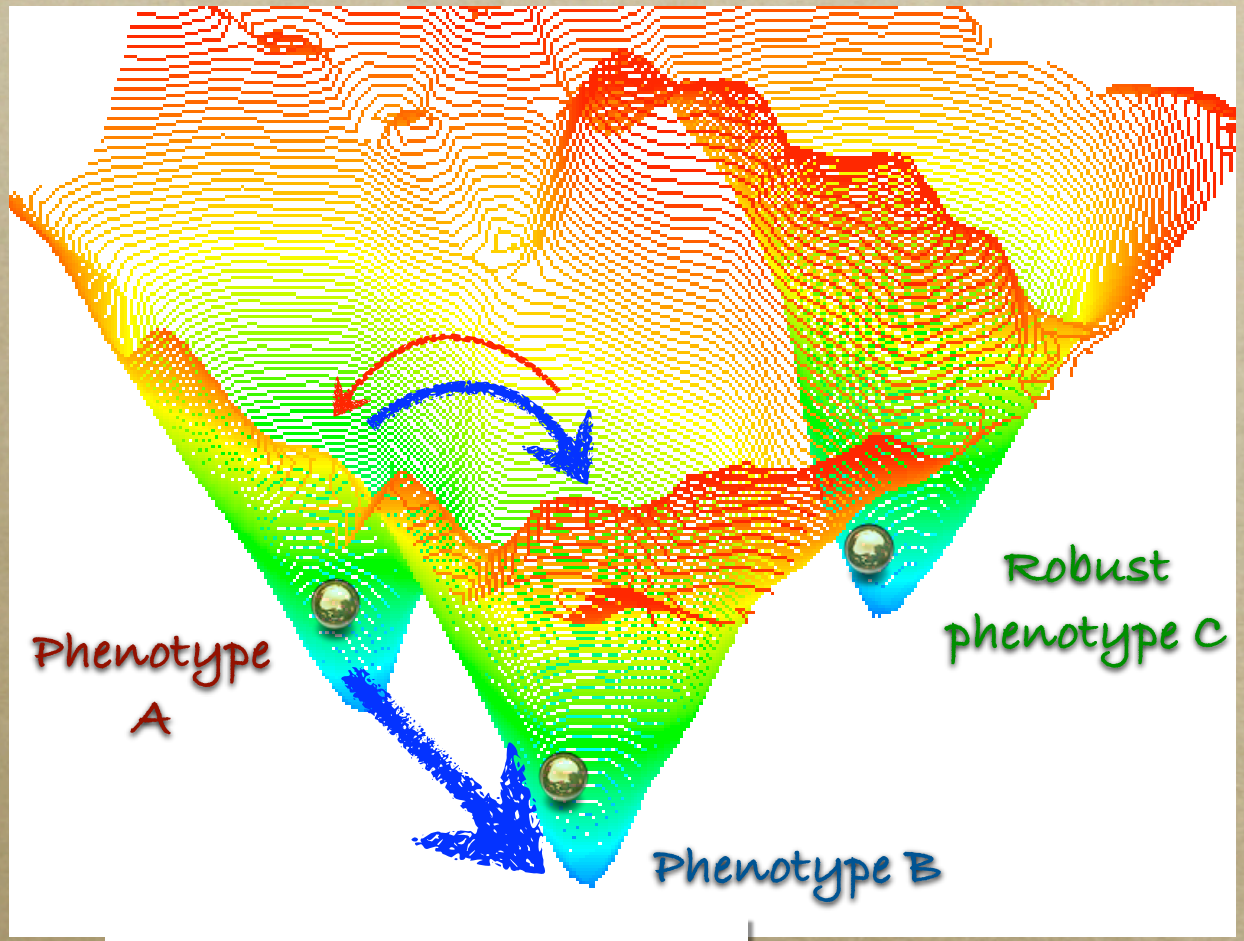


Image by Lei Yuan

- 2 mutually exclusive phenotypes in 1 population

-> advantage under abrupt environmental change

Consequences of noise in response to a cellular signal



signal promoting phenotype B

- Whenever a signal needs to flip a bistable switch (cross a barrier)

- Intermediate strength: noise matters

- Is there a biological reason to do this, on purpose?

A Two-Dimensional ERK-AKT Signaling Code for an NGF-Triggered Cell-Fate Decision

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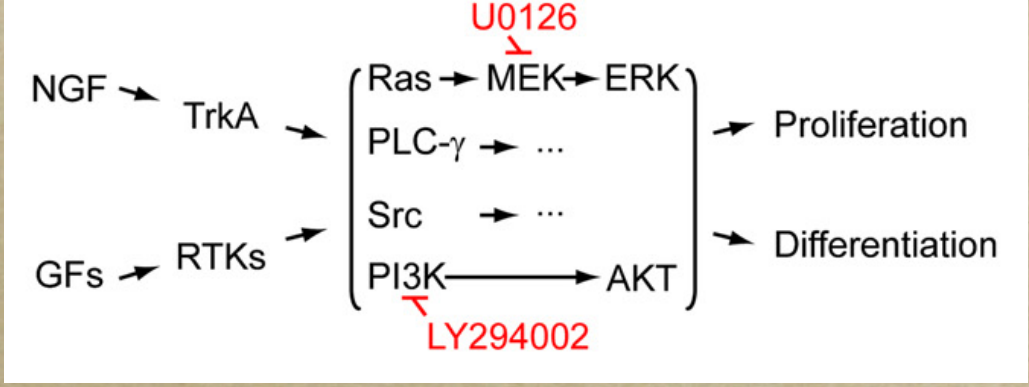
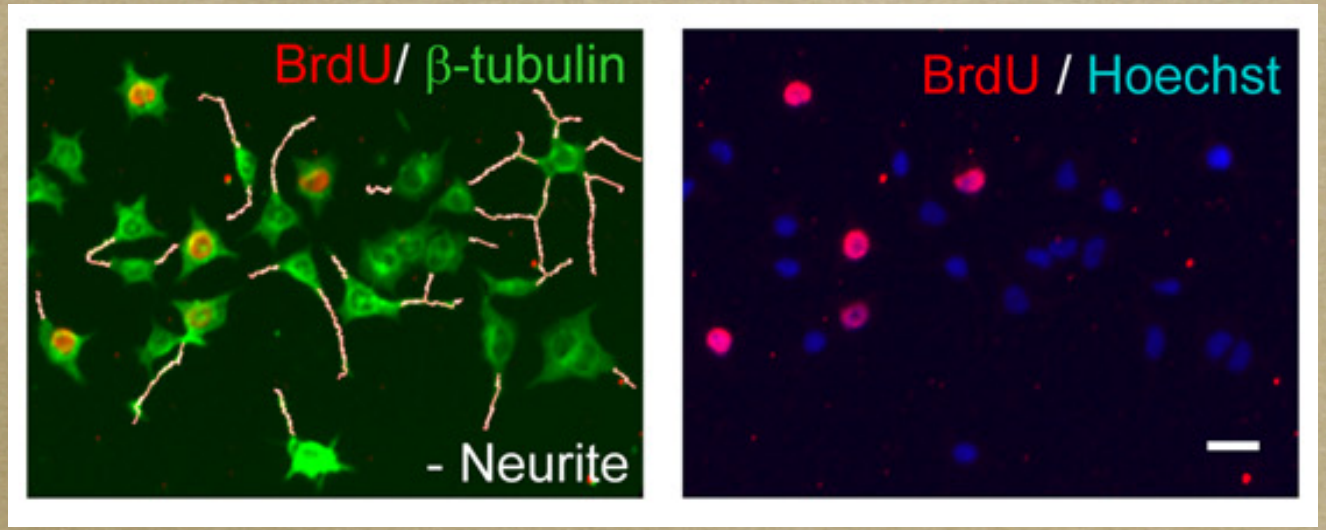
PC12 cells can undergo neuronal differentiation, or remain in a proliferative, stem-like state

Complex pathways → **?** → **Cell fate decision**

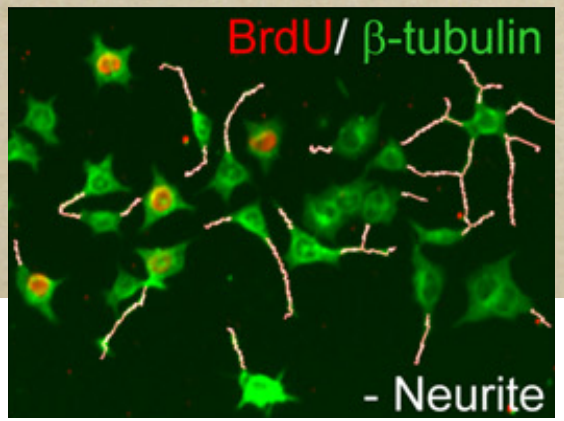


PC12 - cell line from pheochromocytoma rat adrenal medulla

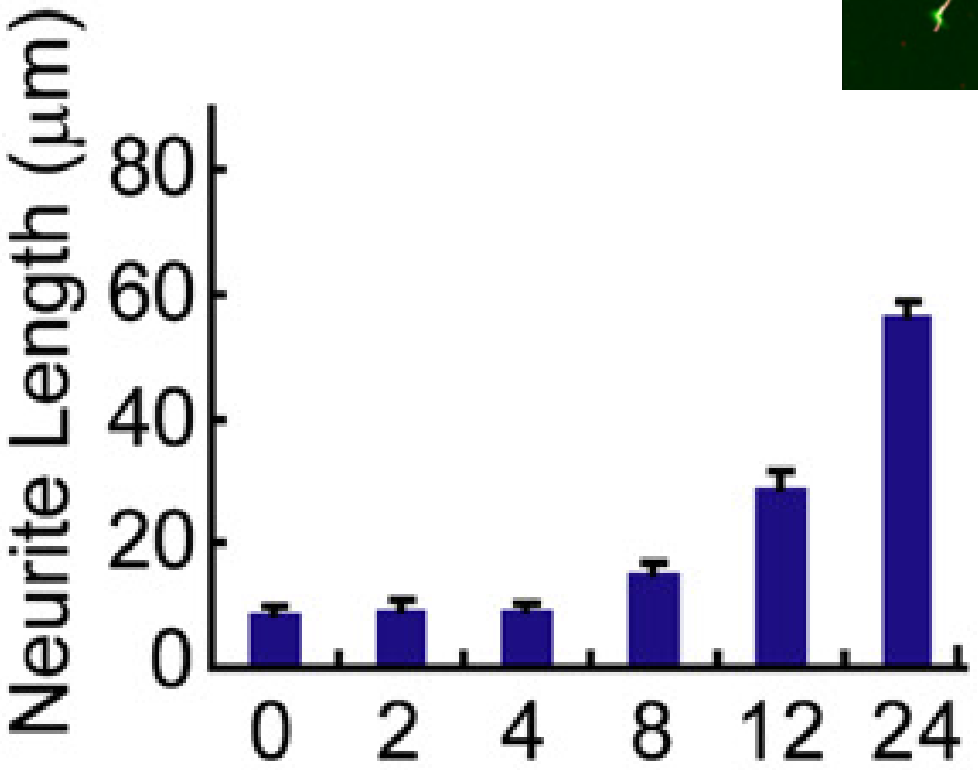


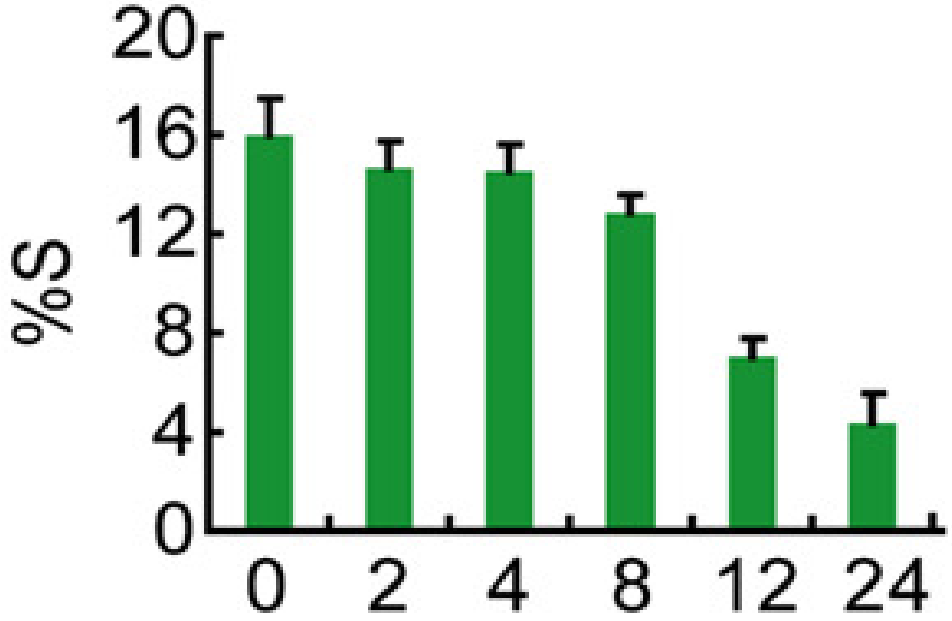
NGF triggers terminal differentiation in most cells

neuron-line morphology



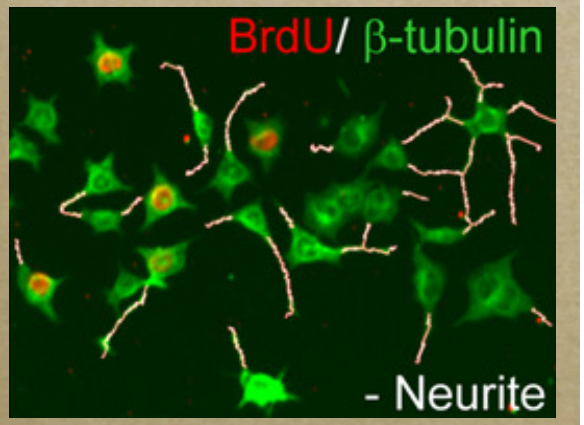
cells in S phase

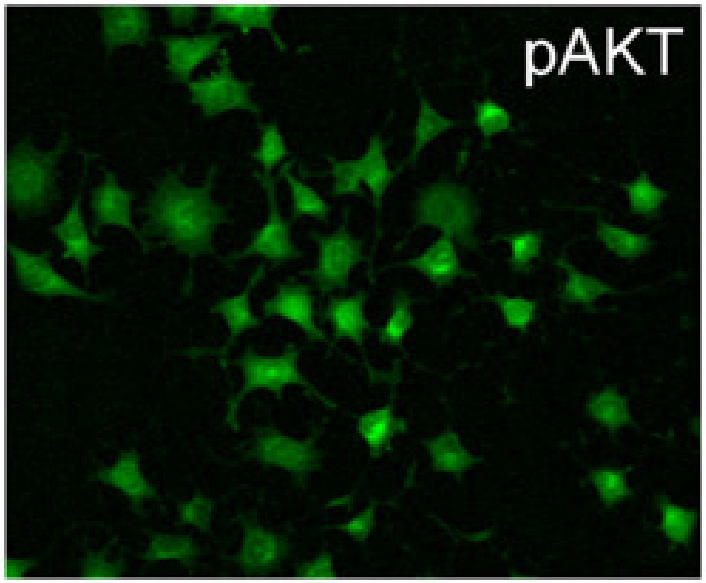


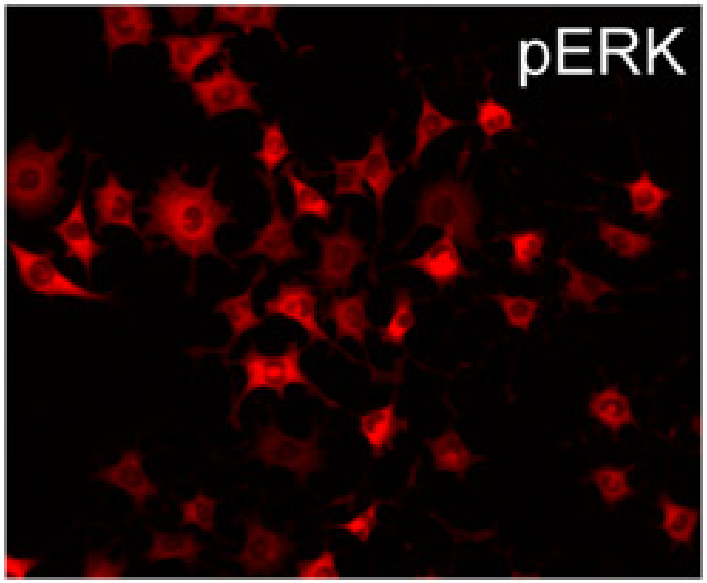


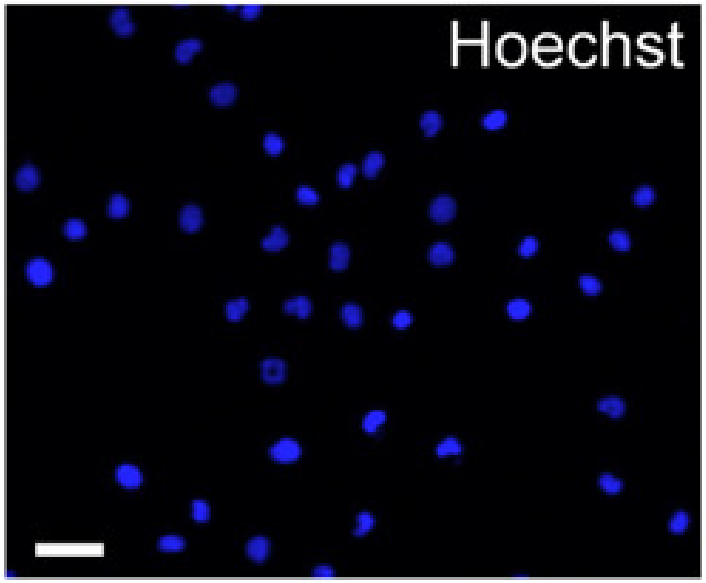
Time after NGF (hr)

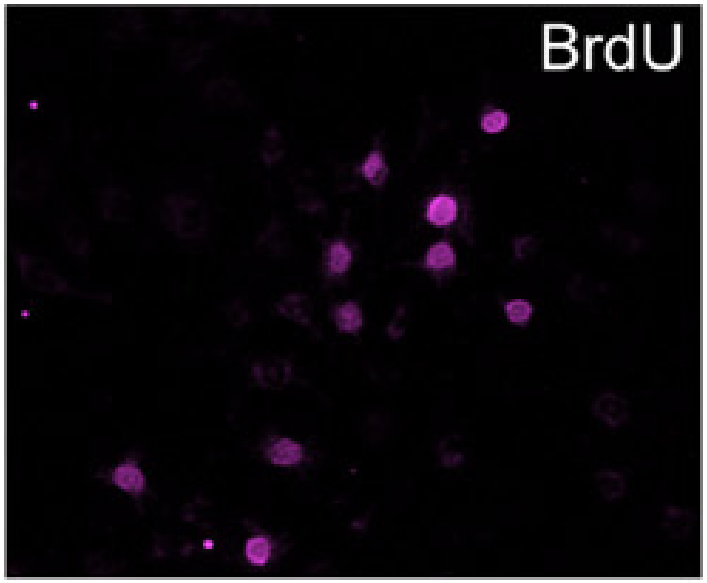
Main assay - pERK, pAKT, BrdU & Neurite in single cells







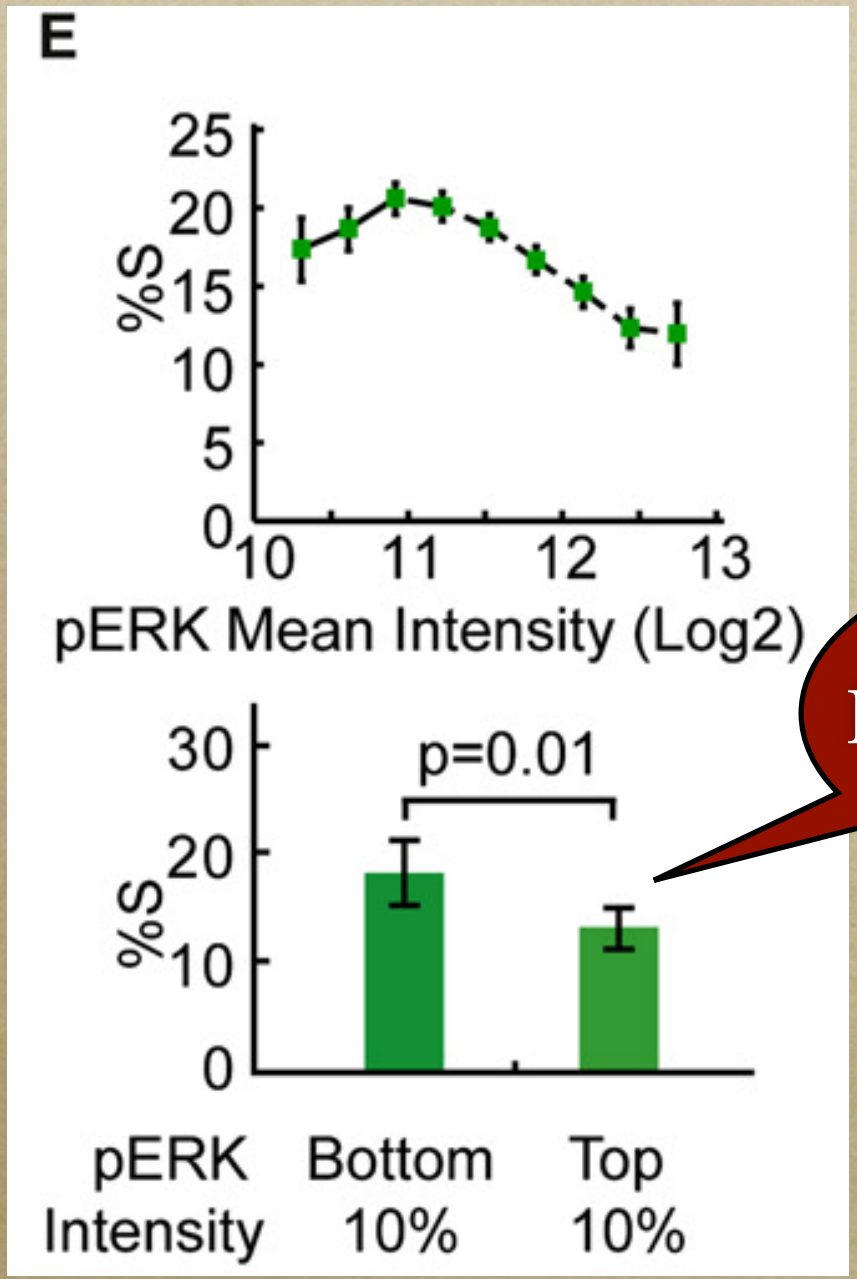




pERK signal strength is a poor predictor of differentiation

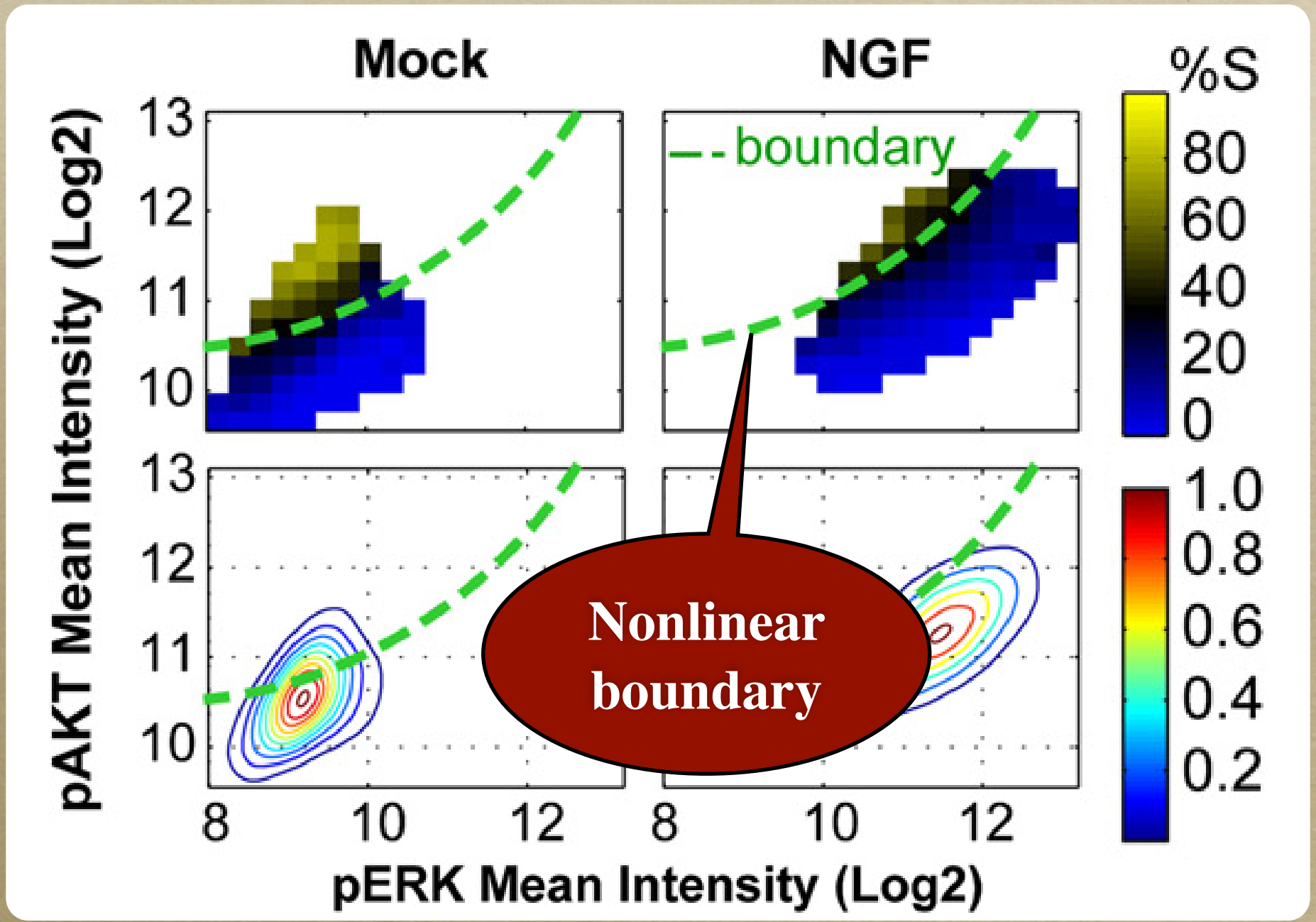
Literature:
sustained pERK
<=>
differentiation

Populatio n assays

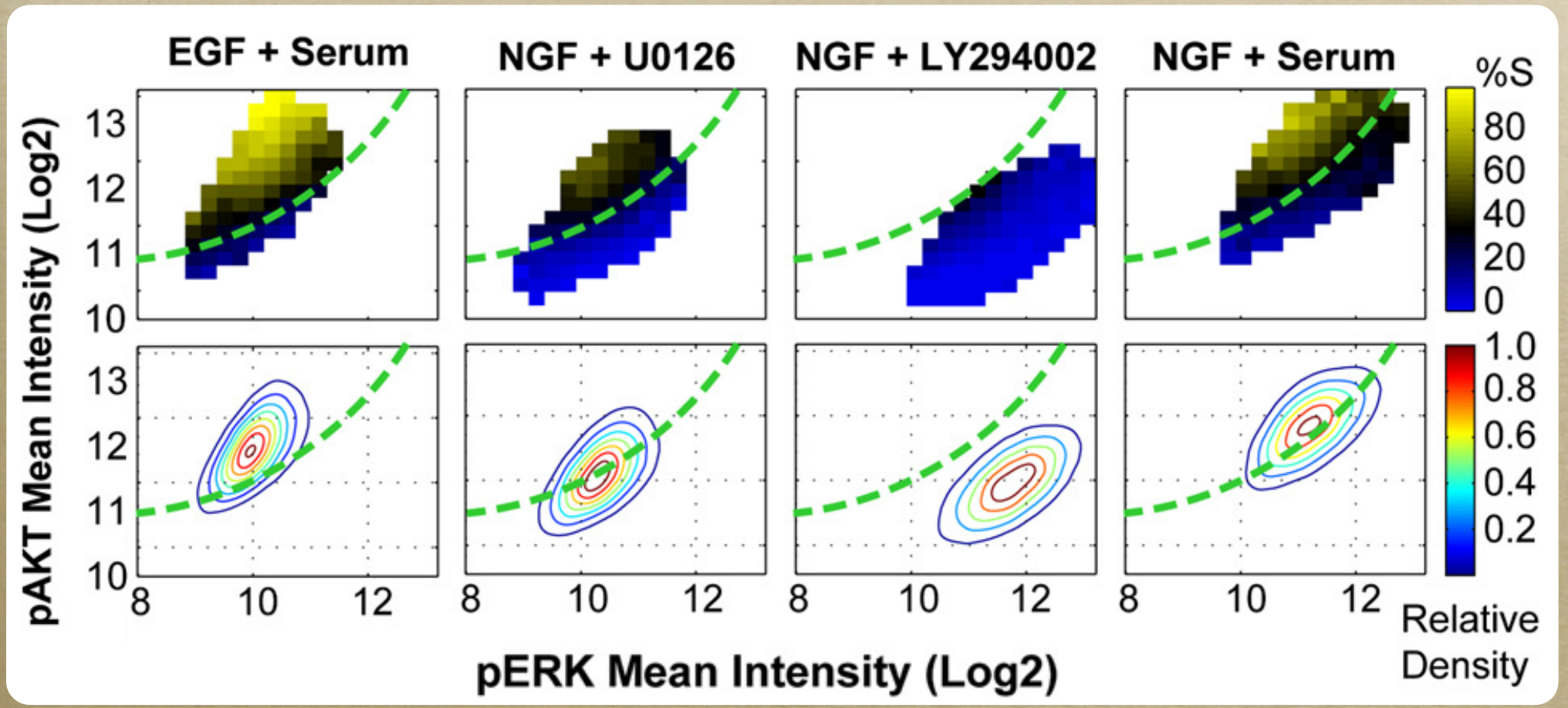


Individual cells!

2D map of pERK-pAKT signal is an excellent predictor of cell fate



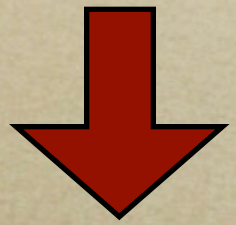
Boundary is sharp, and independent of upstream signals



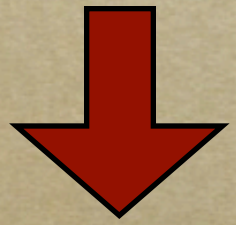
Cell population responds differently, but the boundary does not shift

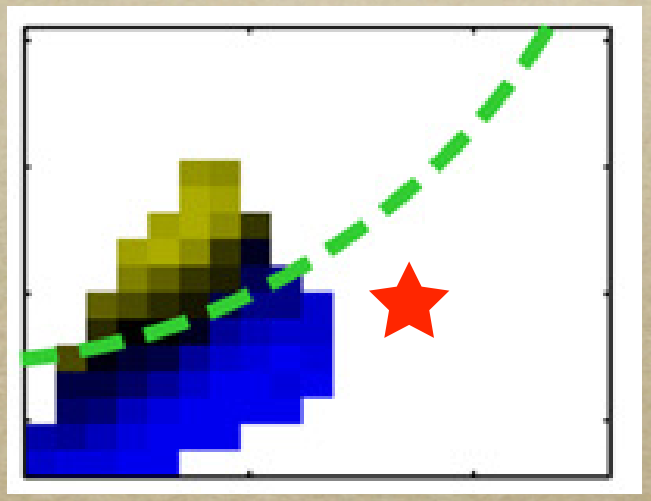
Take-home 1.

Any point of 2D map (or phase space)



Distance from boundary

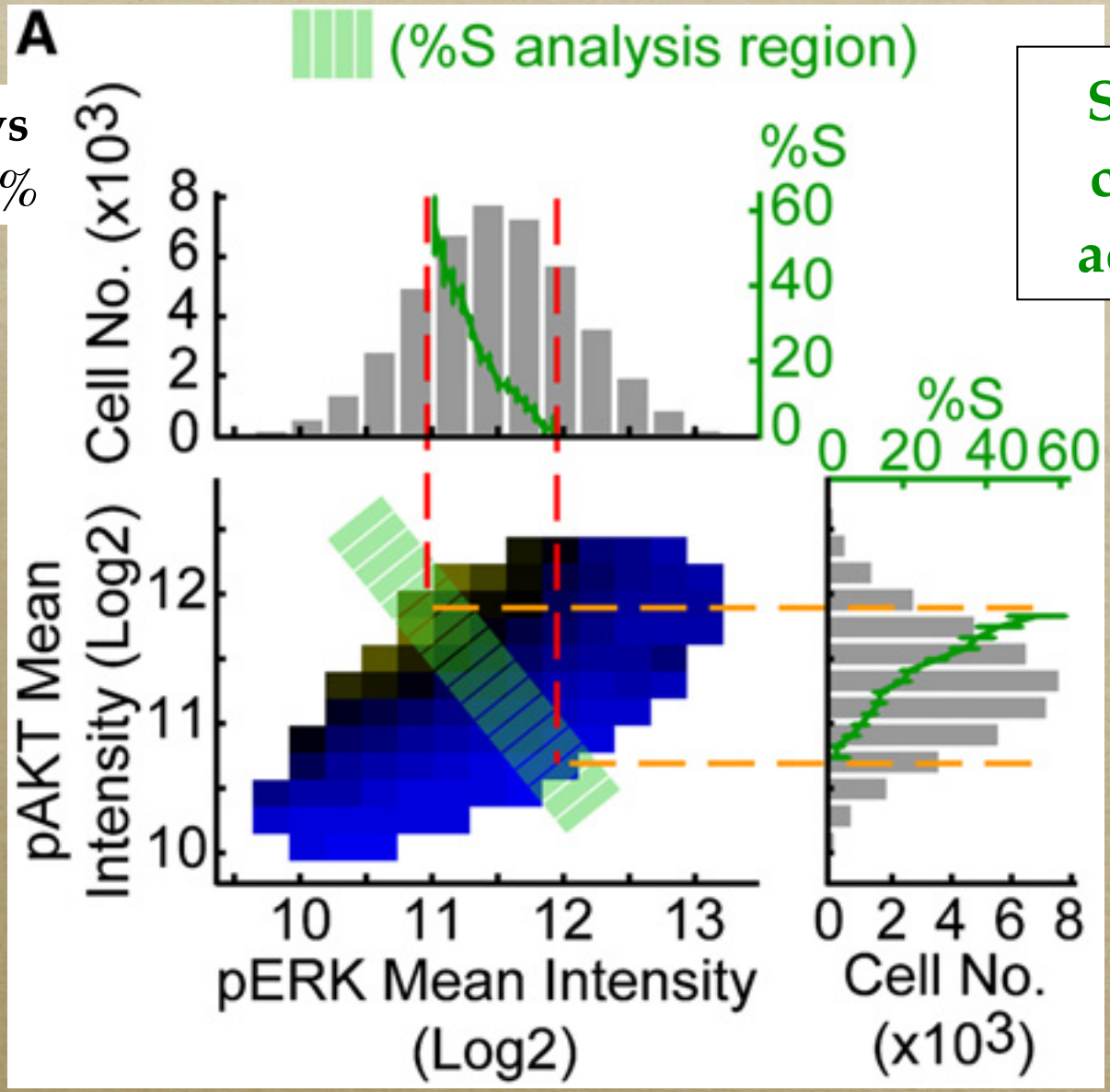




Probability of proliferation vs differentiation

Population shows marked cell-to-cell heterogeneity in pAKT and pERK response

top 5% vs bottom 5%



pAKT - 3.1-fold

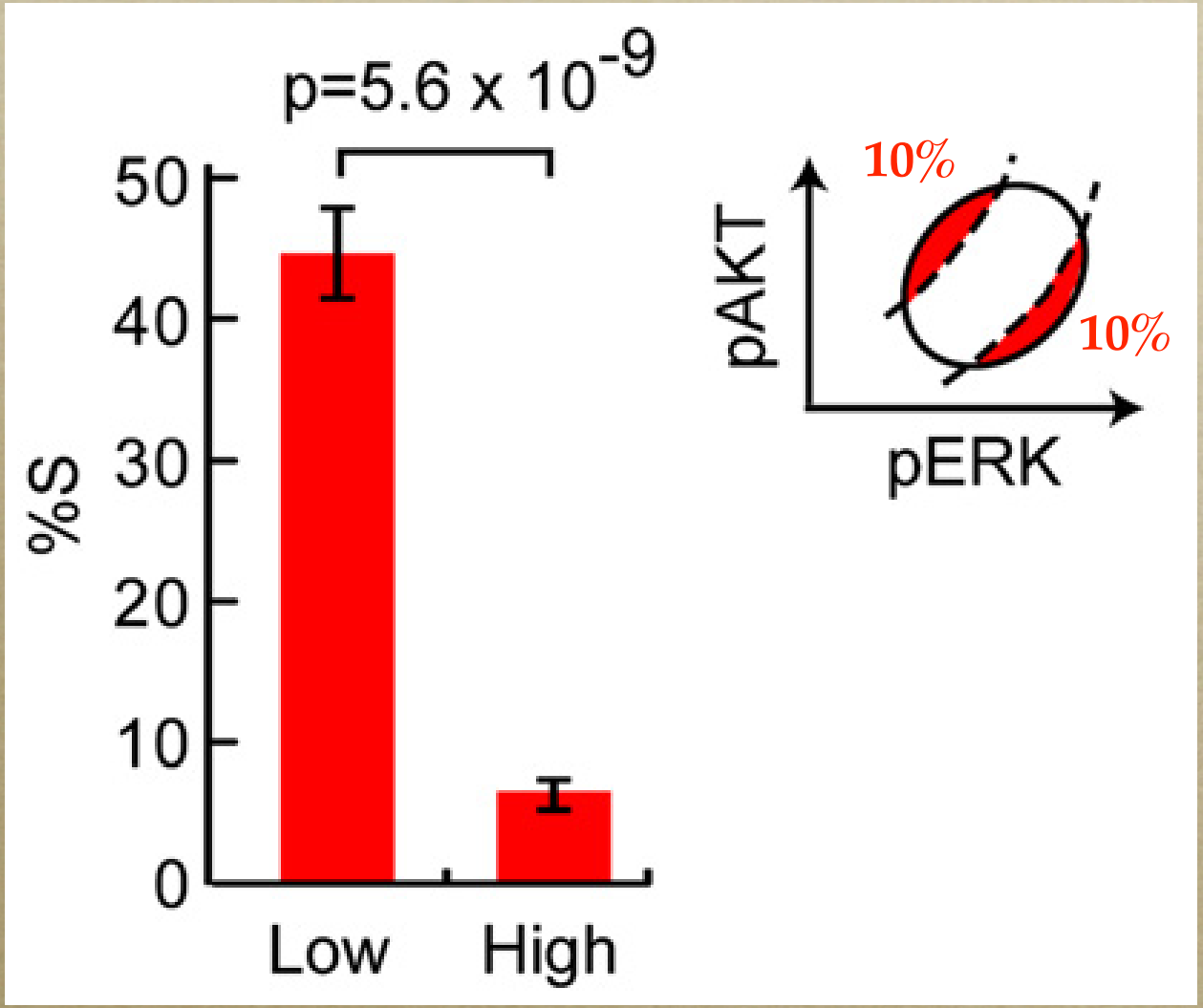
pERK - 4.2-fold

Steepness: % of cells in S phase across boundary

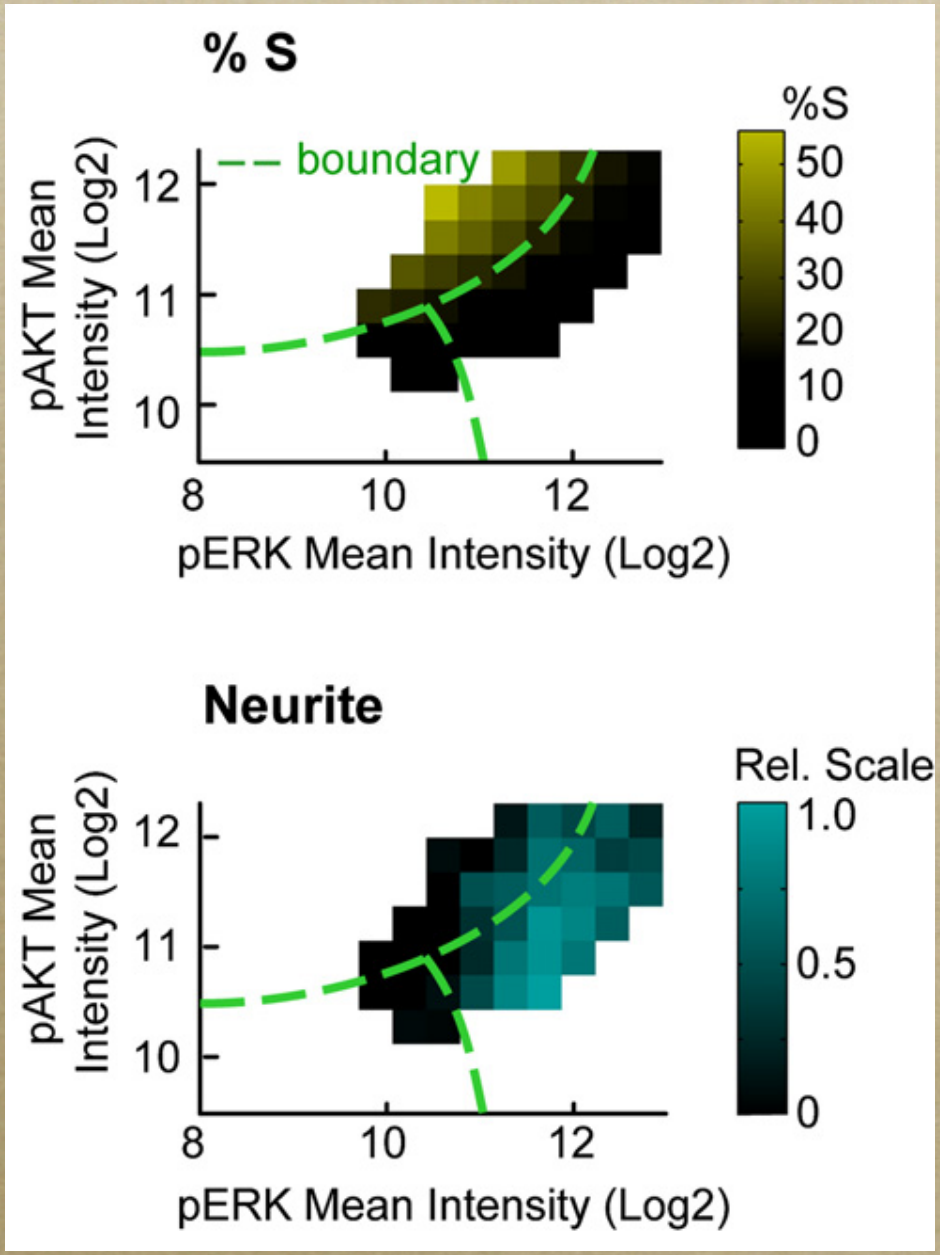
2-fold change in pAKT/pERK

30-fold increase in proliferation

Cells with a large distance from boundary have predictable fates

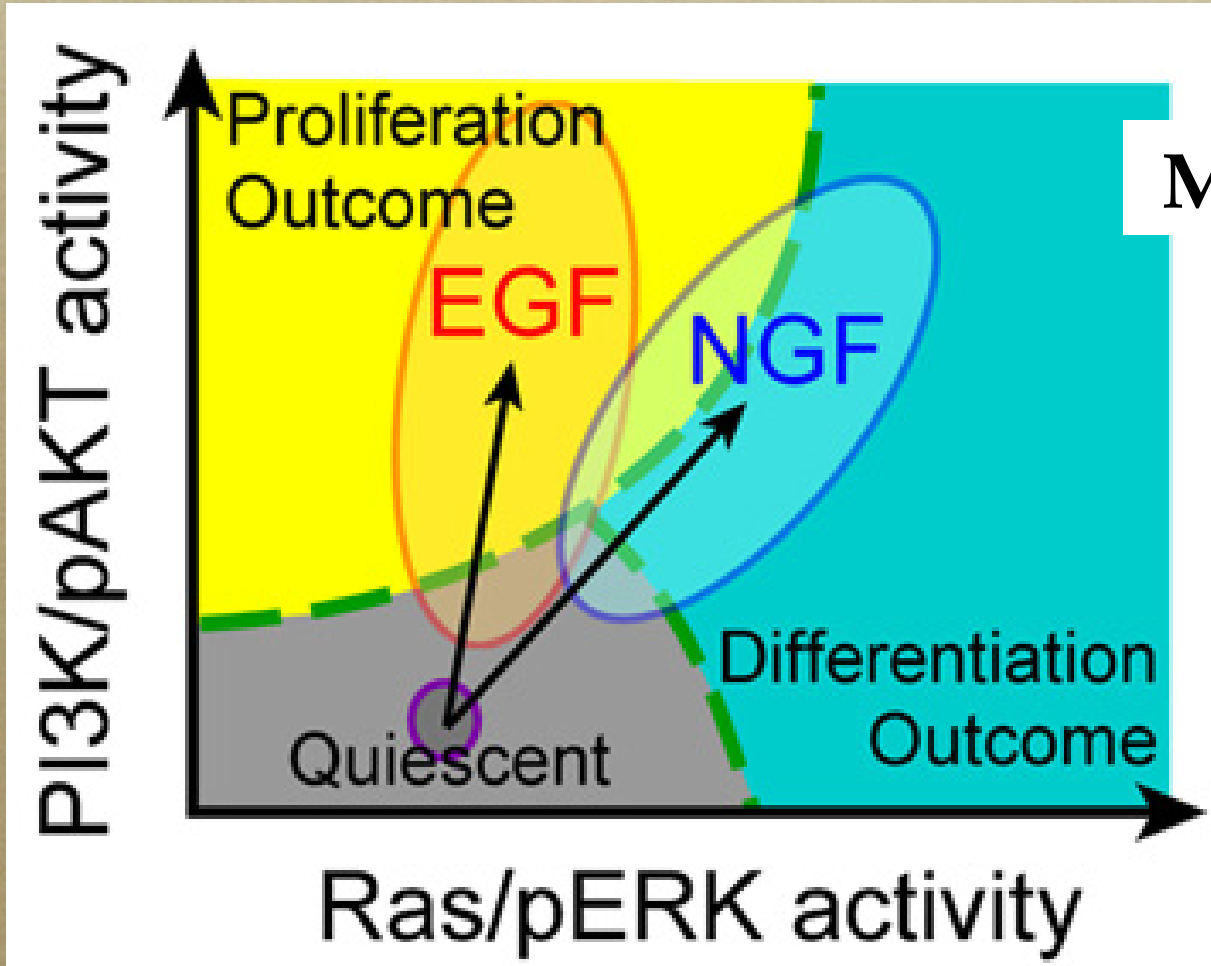


Proliferation and differentiation are mutually exclusive



Take-home II.

Different inputs move quiescent cells onto distinct regions of the 2D map



Model

Linear pAKT / pERK ratios are weak predictors

HOW ?

siRNA screen can probe the underlying circuit

Generate rat siRNA library (1308 genes)

↓

Transfect PC12 cells w/ siRNA

↓ 24 hr

Wash and add 25ng/ml NGF

↓ 48 hr

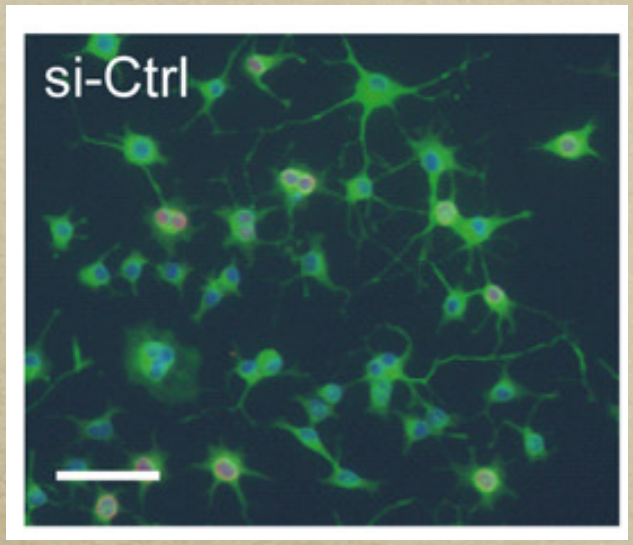
Pulse cells with BrdU for 4hr

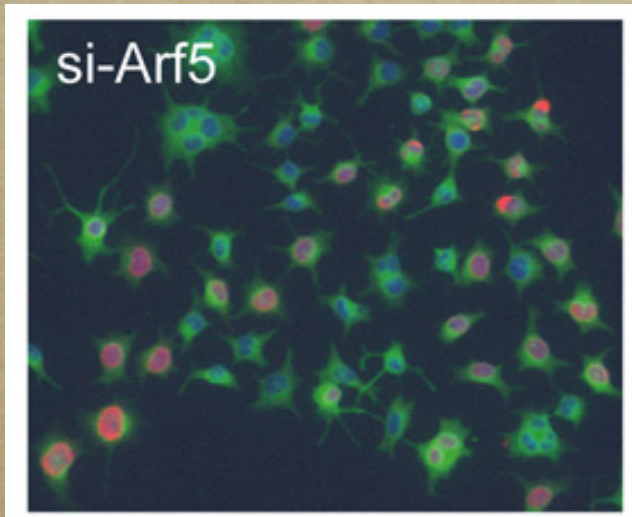
↓

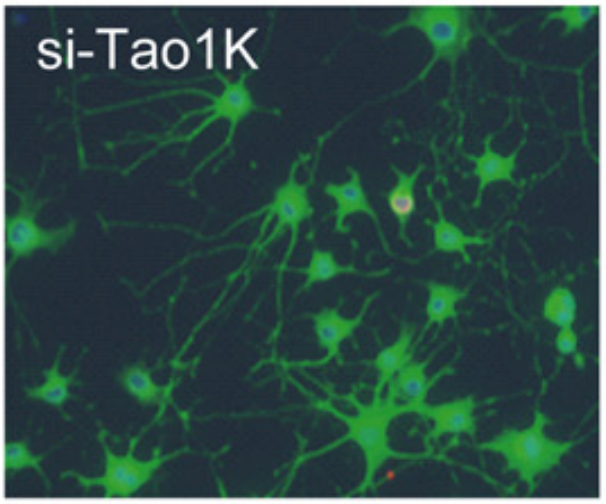
Fix and stain with α-BrdU & α-tubulin βIII Ab

↓

Select hits and validate the hits with different siRNAs



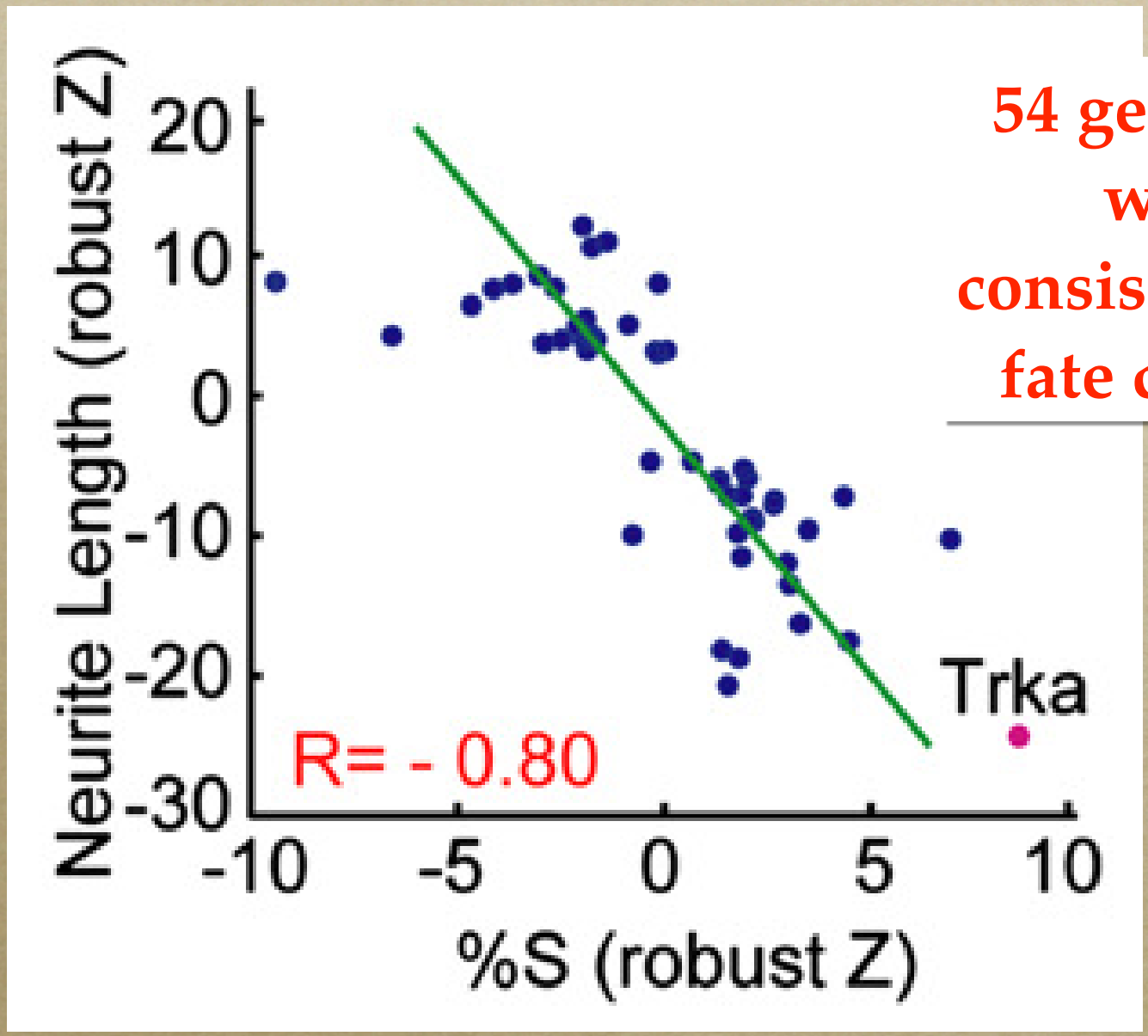




Proliferation

Differentiation

Proliferation and differentiation are tightly coupled (change in concert)

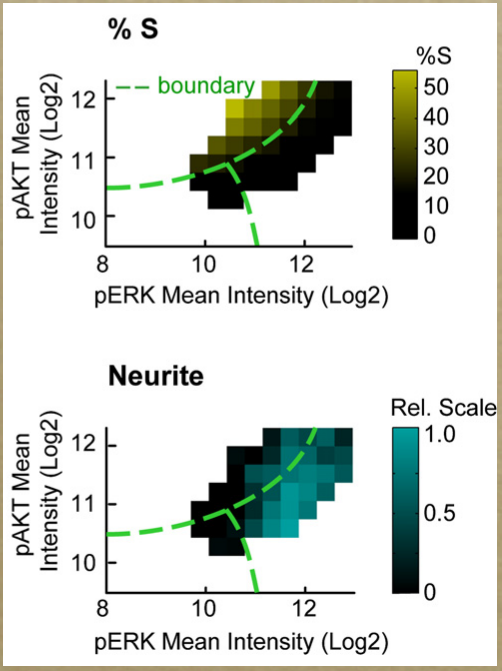


54 gene hits with consistent cell fate change

How early are cell fate decisions predictable from the pAKT/pERK map?

TIME?

Old: all @ 24h

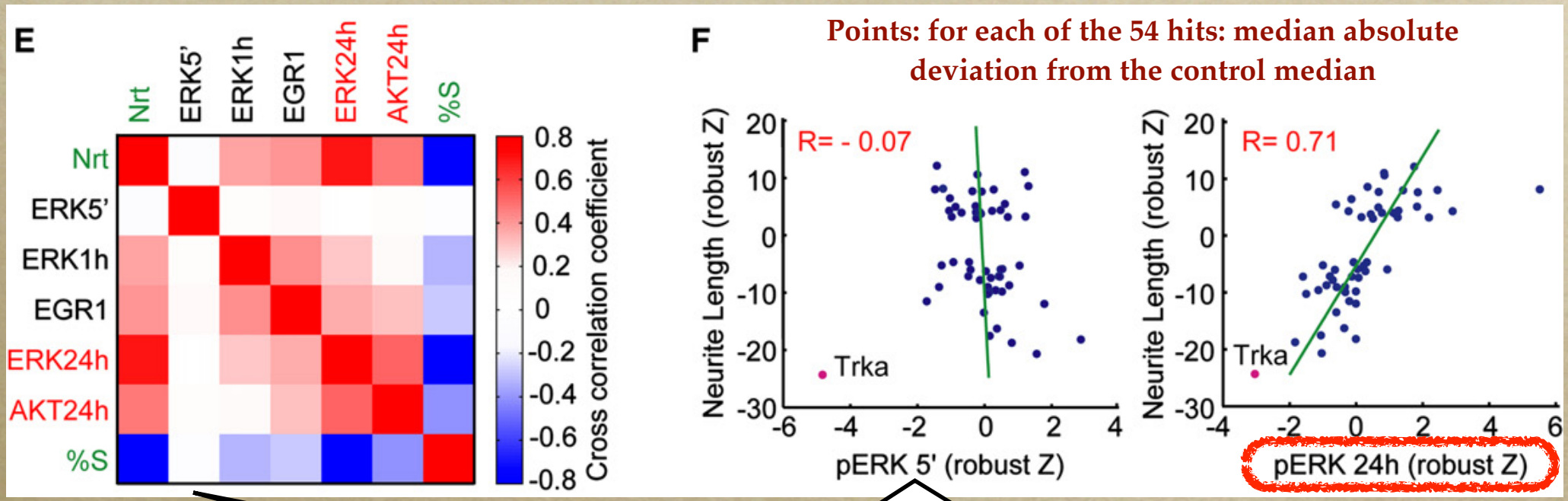


New: pAKT/pERK up to 24h; cell fate @ 48h

NGF signaling scheme	Secondary assays
Ras activation (min)	p-ERK (5min & 1h)
Early gene induction (min - hours)	EGR-1 induction (1h)
Long term signaling (hours - days)	Long-term ERK & AKT phosphorylation (24h)
Neurite growth & Cessation of proliferation	Neurite length (48h) & Percent in S phase (48h)

time

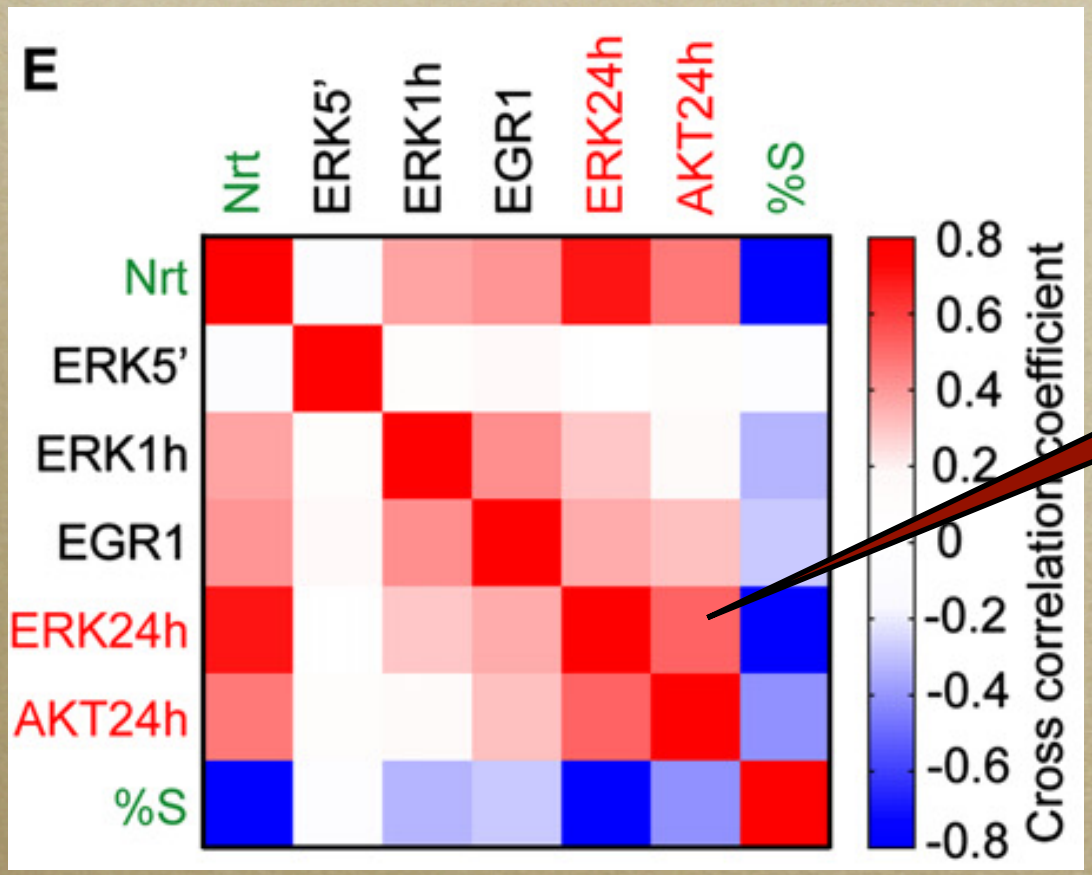
pERK levels at 24 hours predict cell fate at 48h



pERK at 5' has no predictive power

Short-term signals may be altered without changing cell fate, as long as long-term effects remain unchanged.

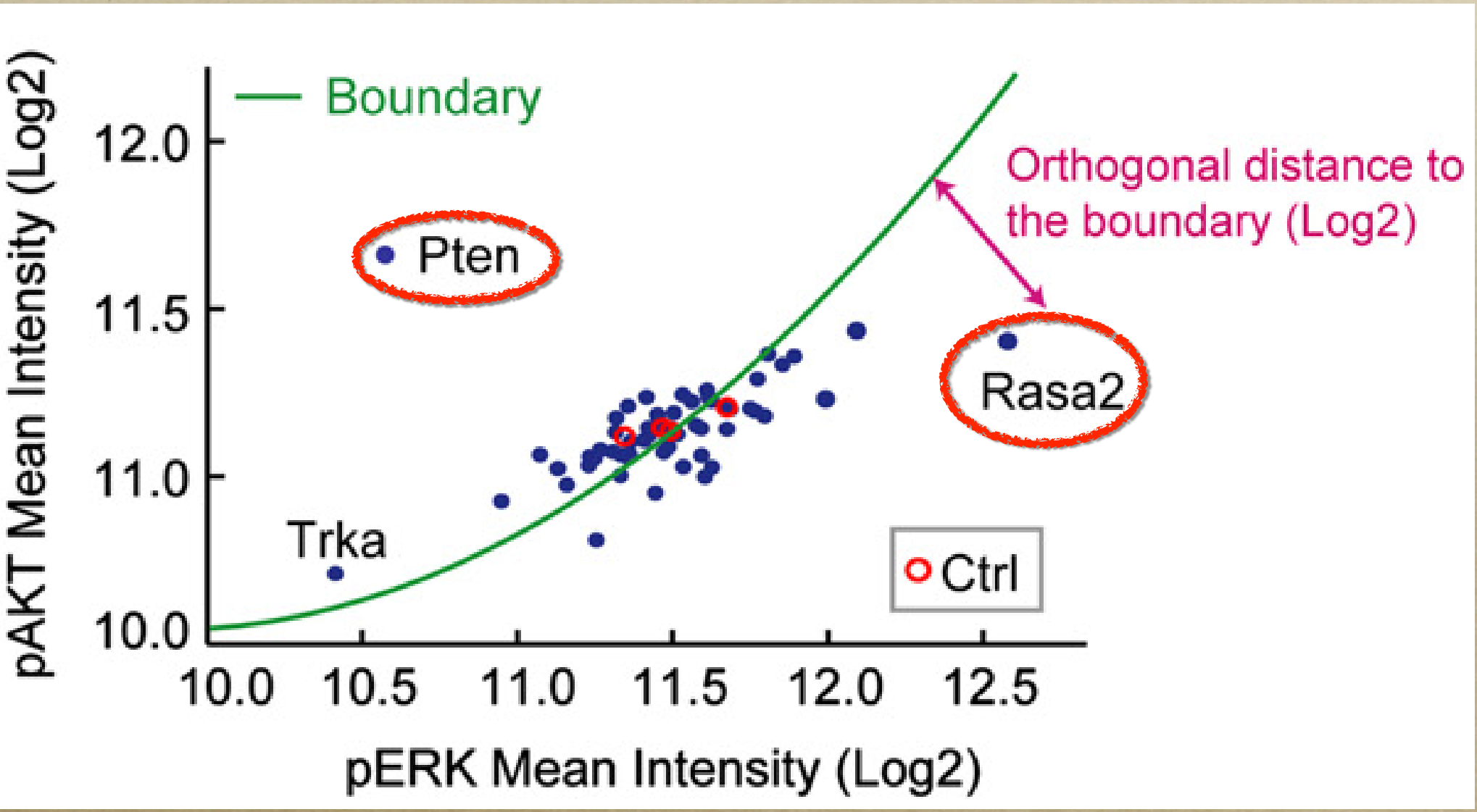
pERK and pAKT are positively correlated at 24 hours



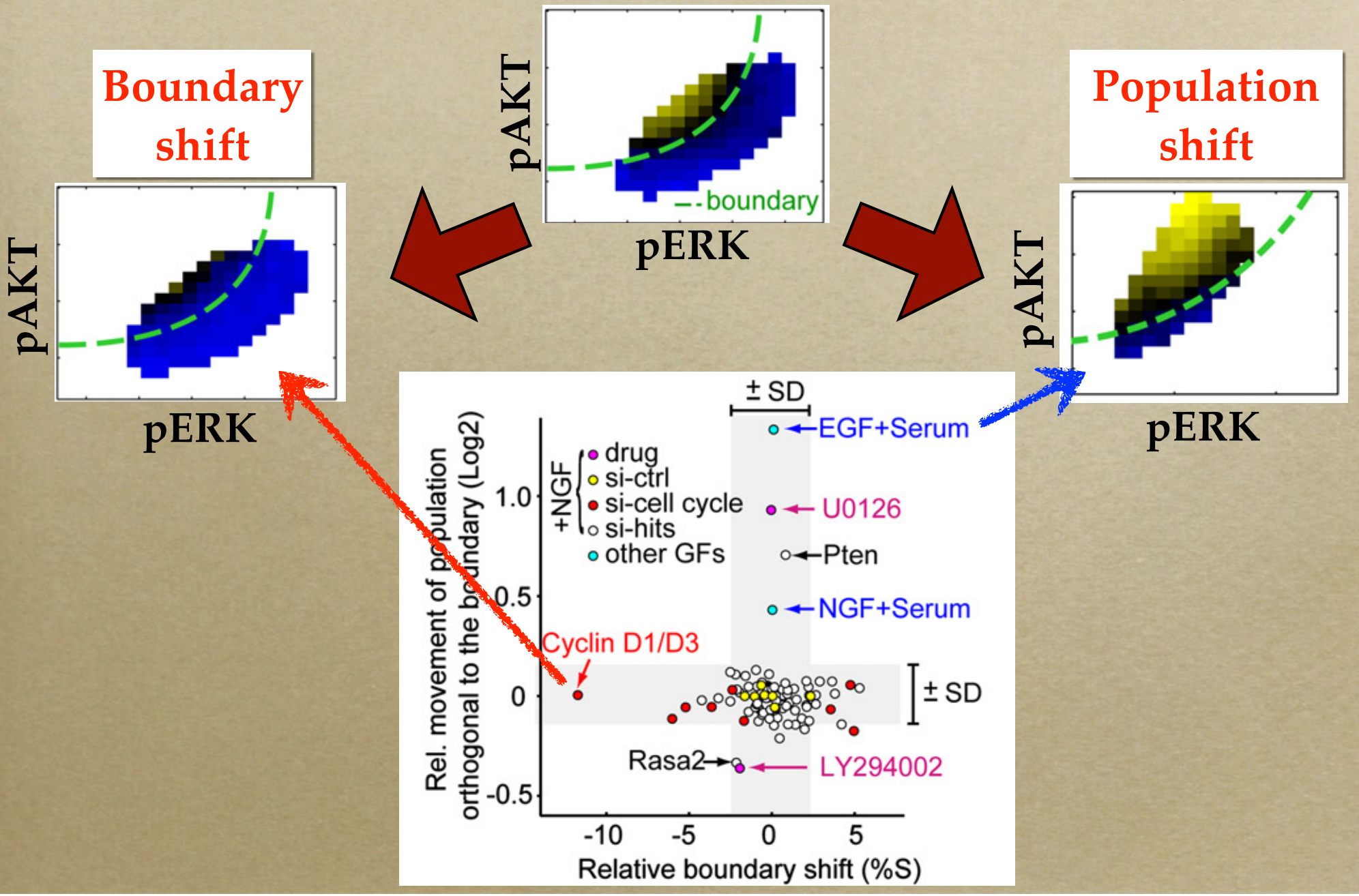
Change in parallel

siRNA perturbations tend not to shift the NGF-treated population far from the (nearly diagonal) boundary

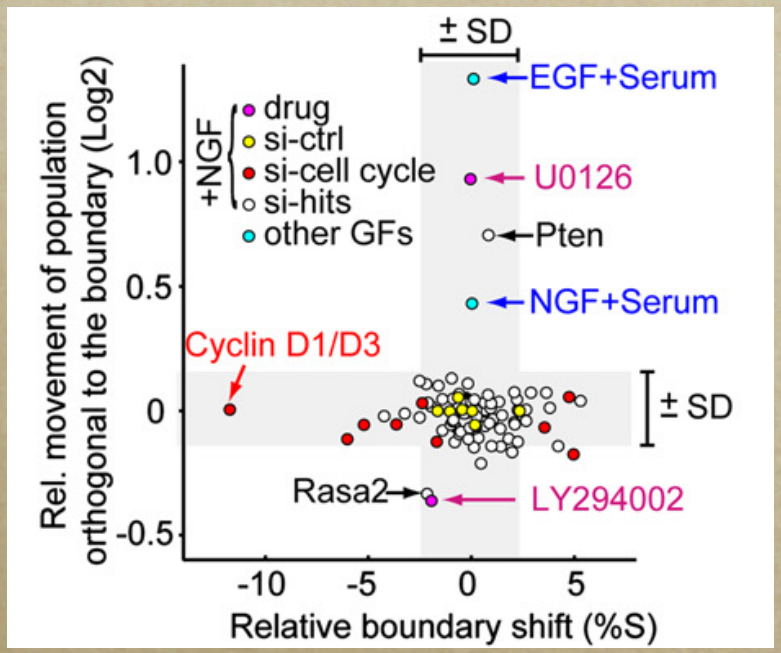
siRNA perturbations rarely shift the NGF-treated population far from the boundary



siRNAs have two distinct effects on the 2D map / cell population relationship



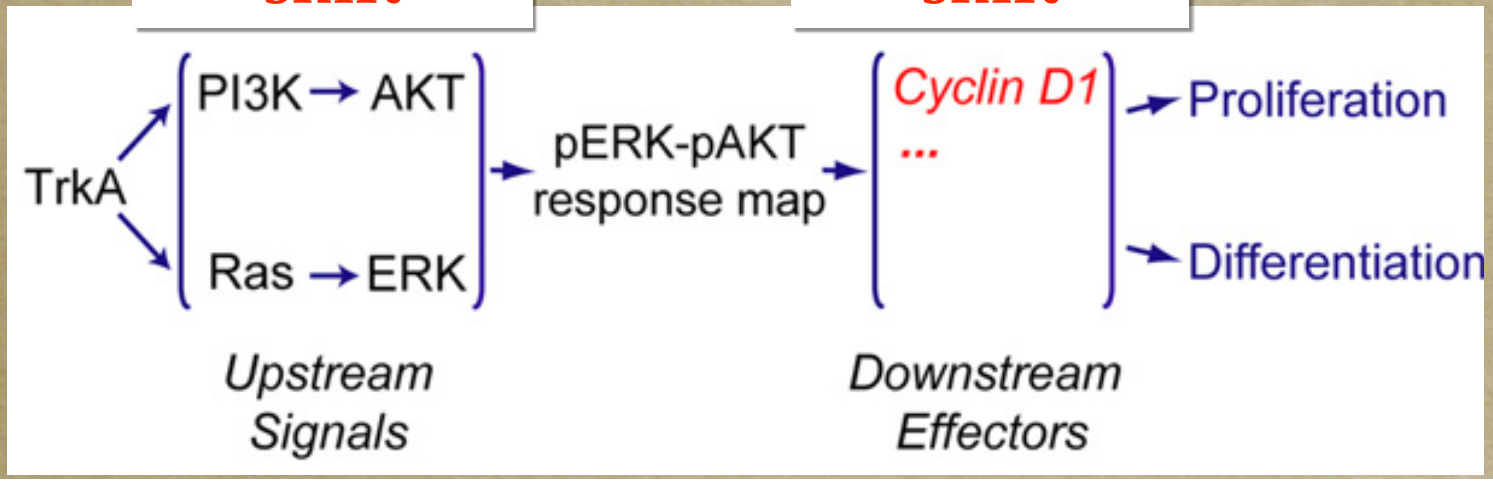
Take-home III.



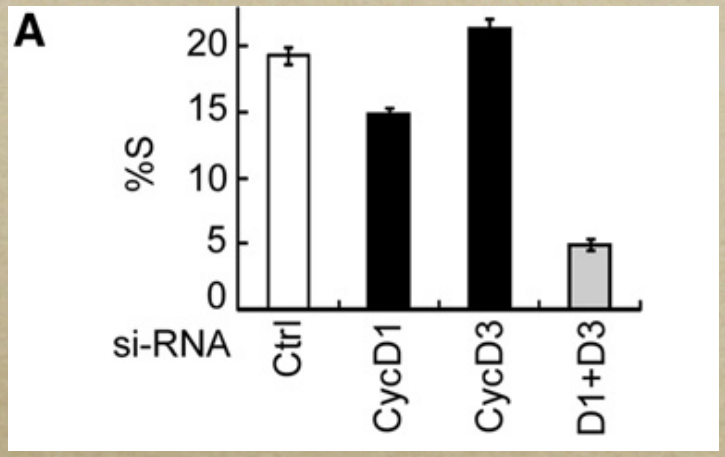
Signaling system has an "hourglass" shape: pERK-pAKT map is the integrating decision point

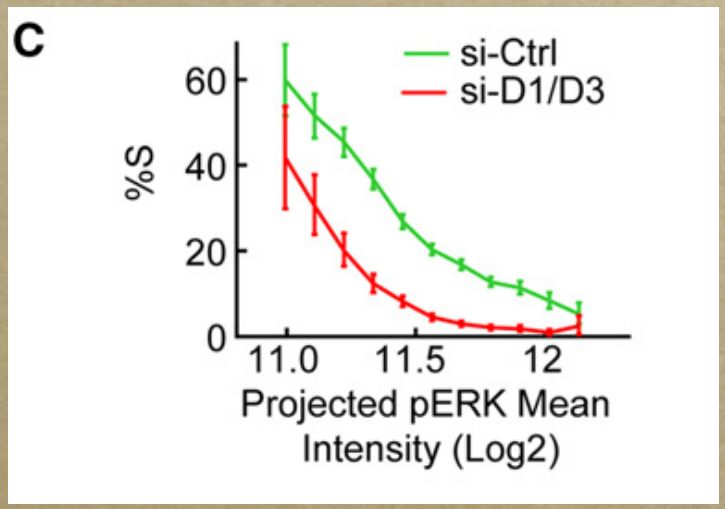
Population shift

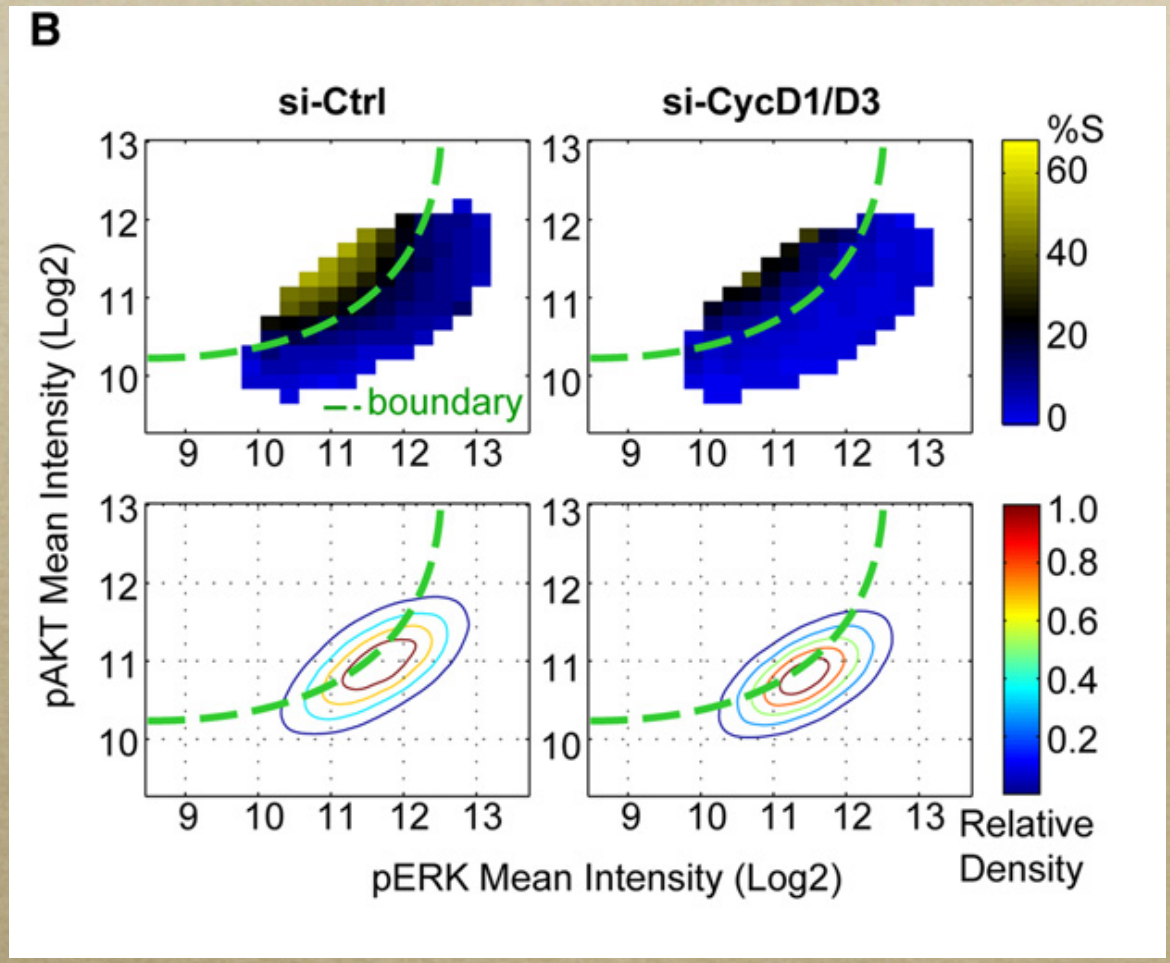
Boundary shift



Downstream - CyclinD1/D3 knockdown induced a strong shift in cell fate boundary

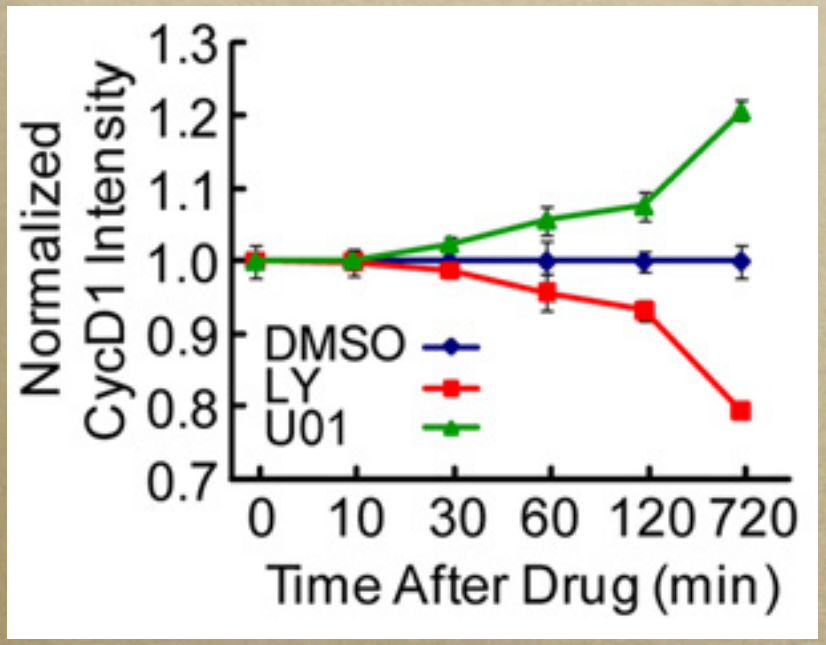


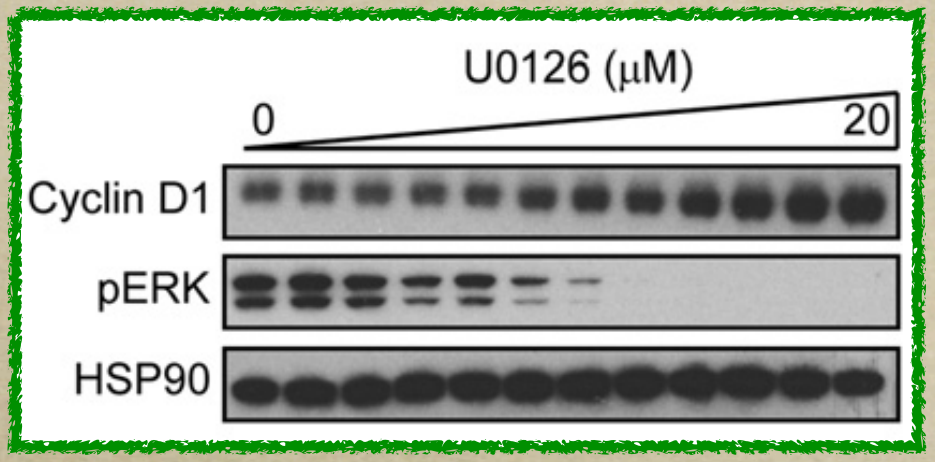




CyclinD1/D3 are critical for transducing the combined effect of pAKT/pERK

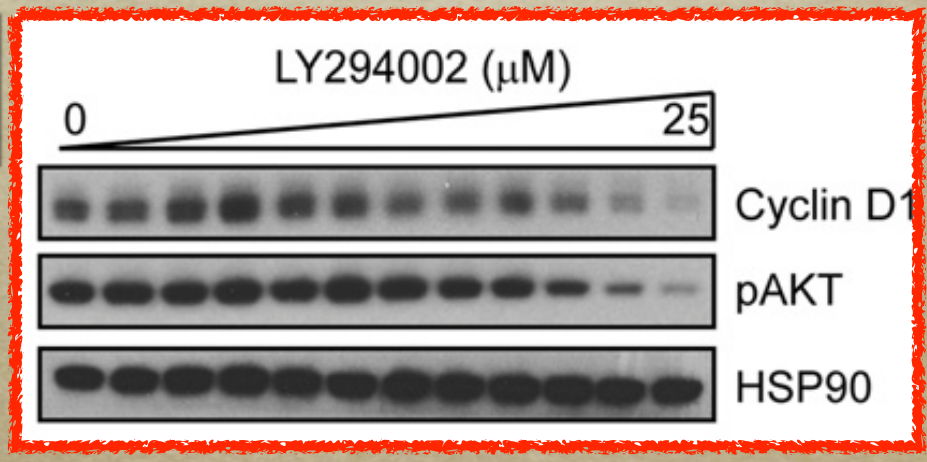
pAKT upregulates, pERK downregulates CyclinD1 protein level



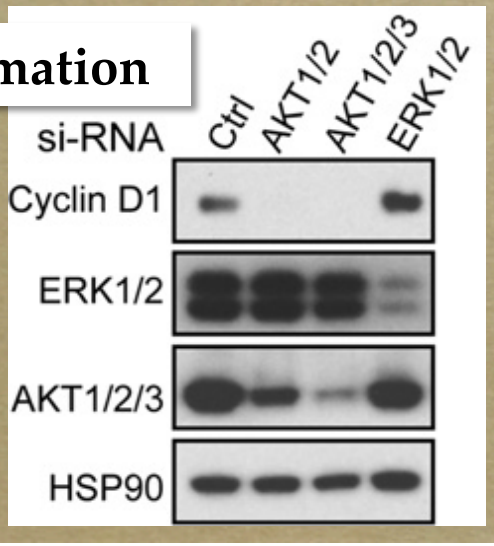


pERK↓

pAKT↓

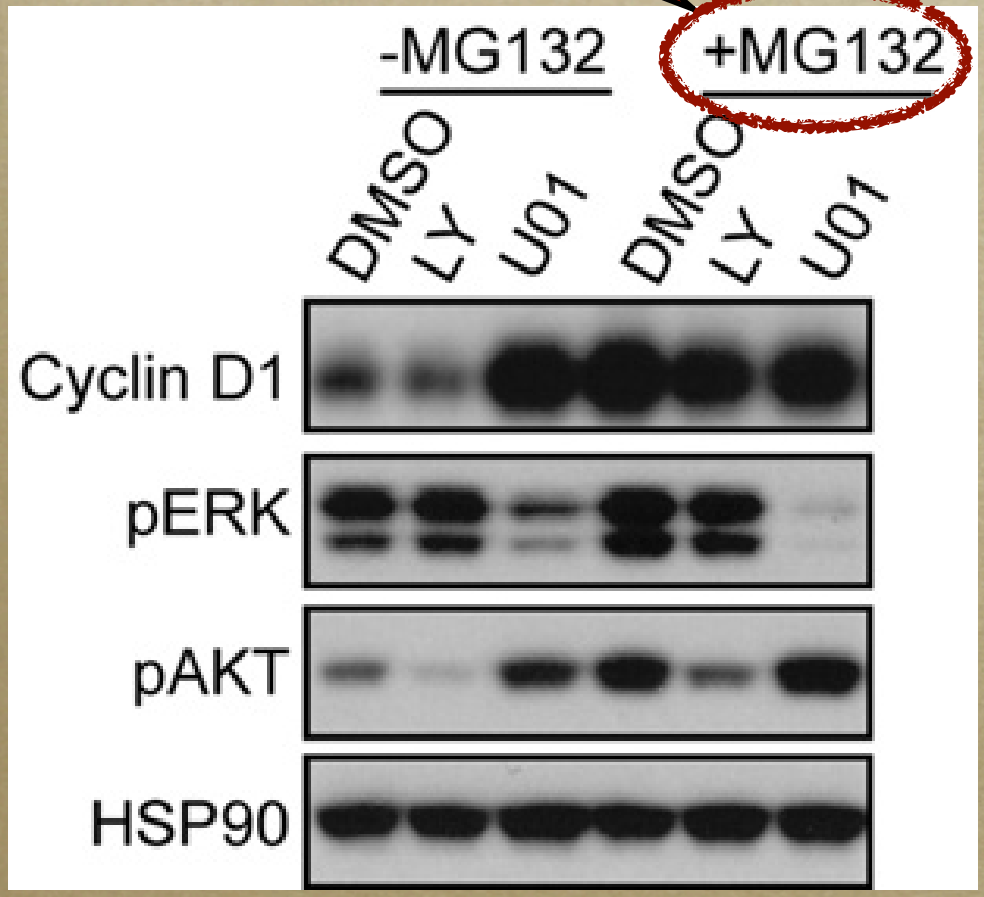


siRNA confirmation

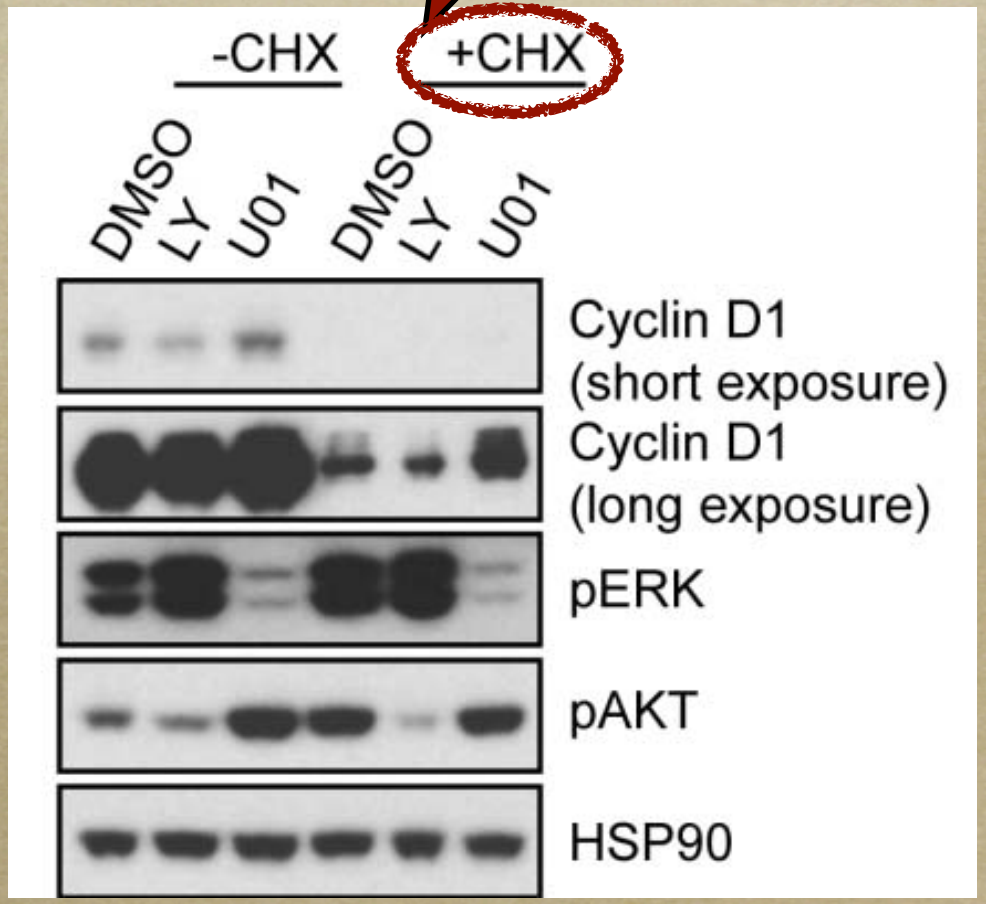


pAKT and pERK affect CyclinD1 protein stability

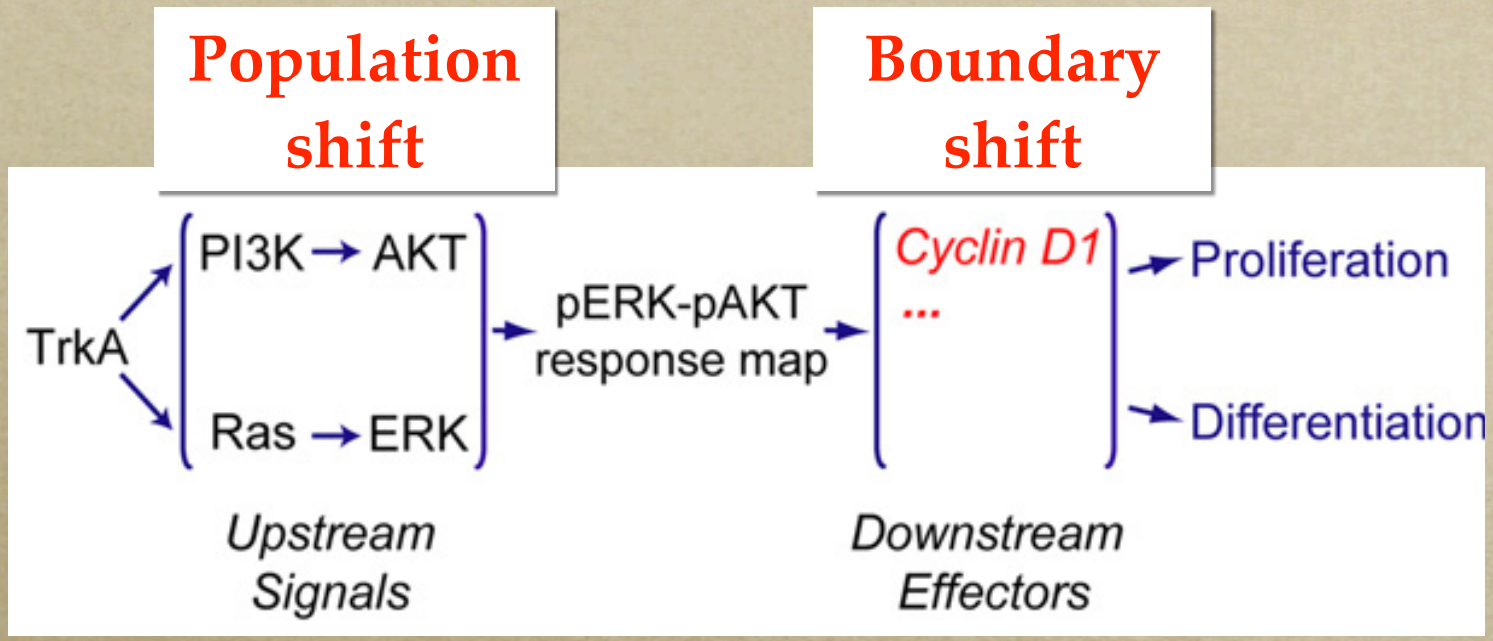
Proteasome inhibitor



Protein translation inhibitor

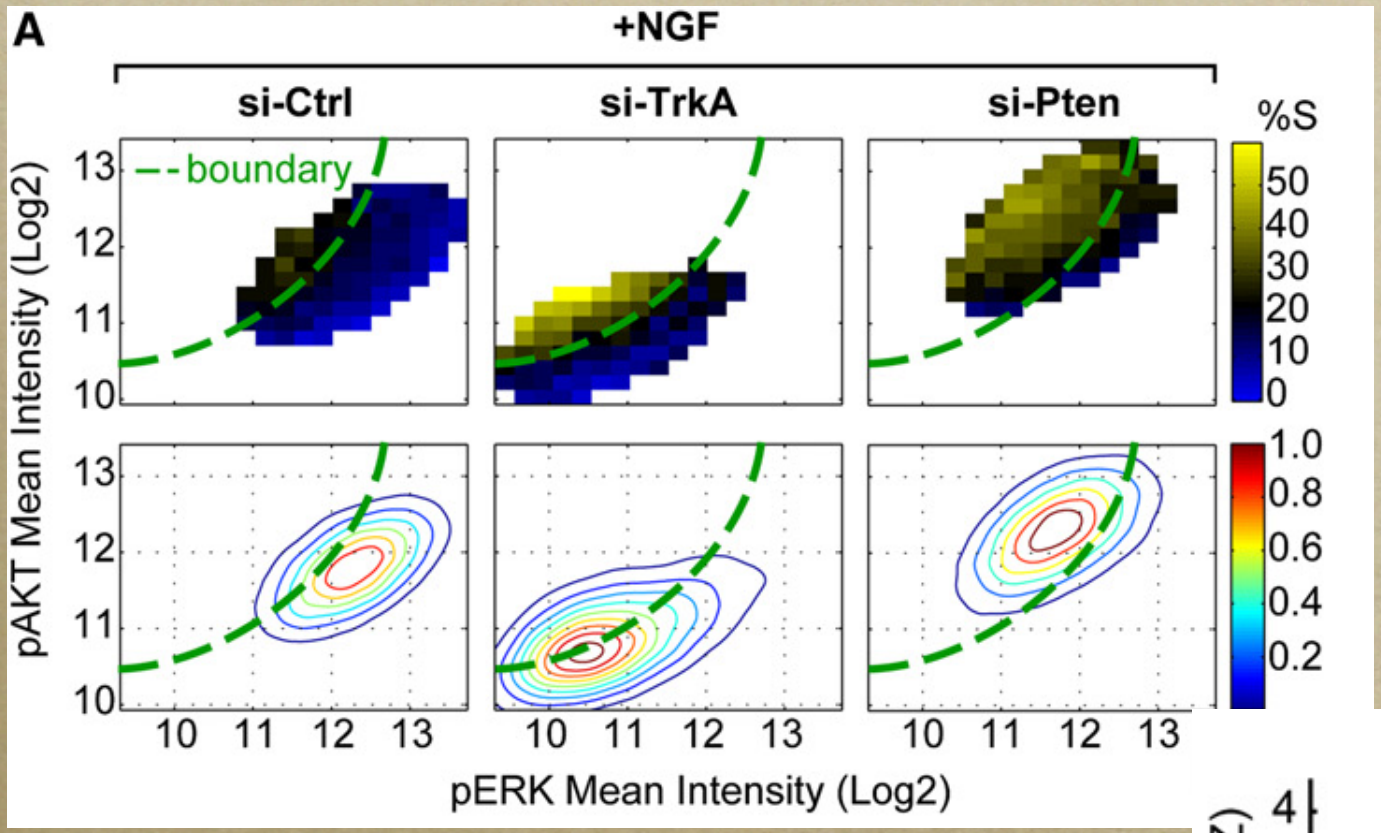


Take-home IV.



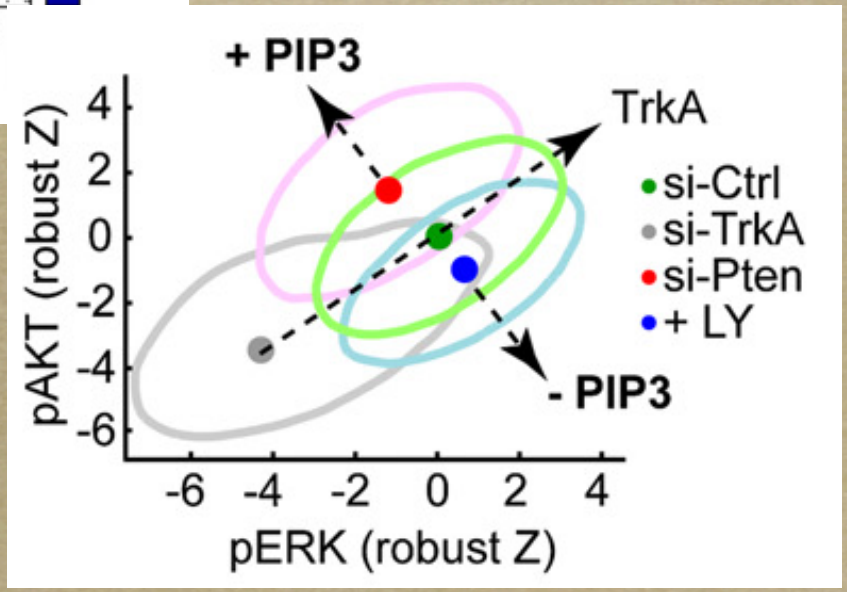
The combined effect of pAKT/pERK translates to proliferation or differentiation by affecting CyclinD protein stability

Upstream - PIP3 shifts the NGF treated population into the proliferation "region"



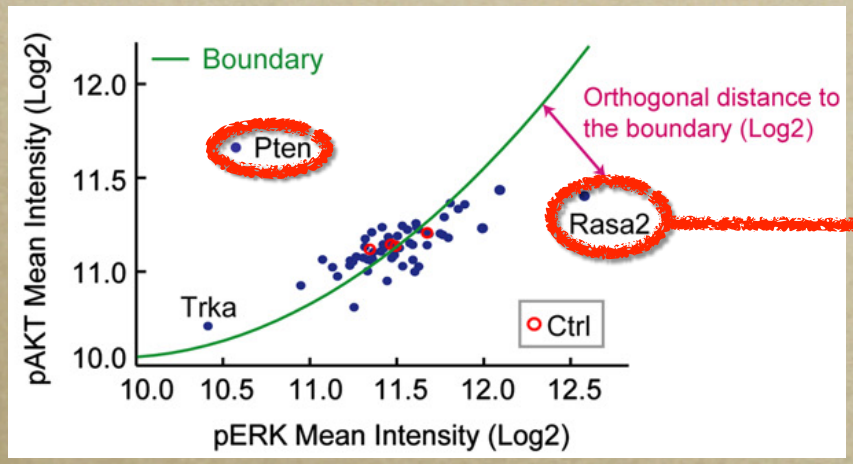
PIP3 regulates the population position orthogonal to the boundary

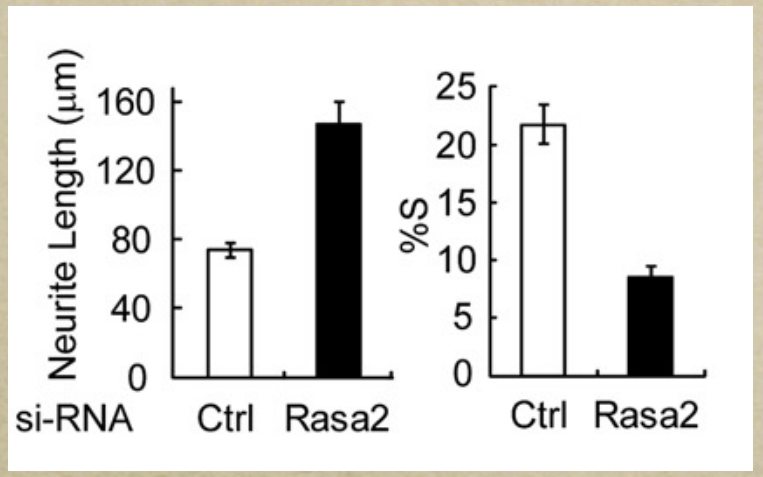
NGF and siTrkA shift the population parallel to the boundary

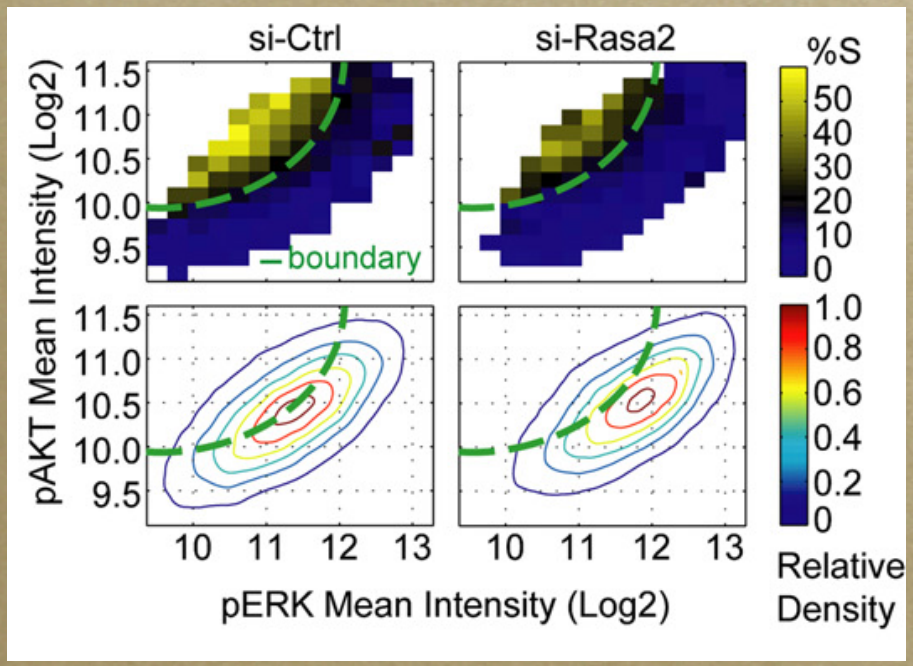


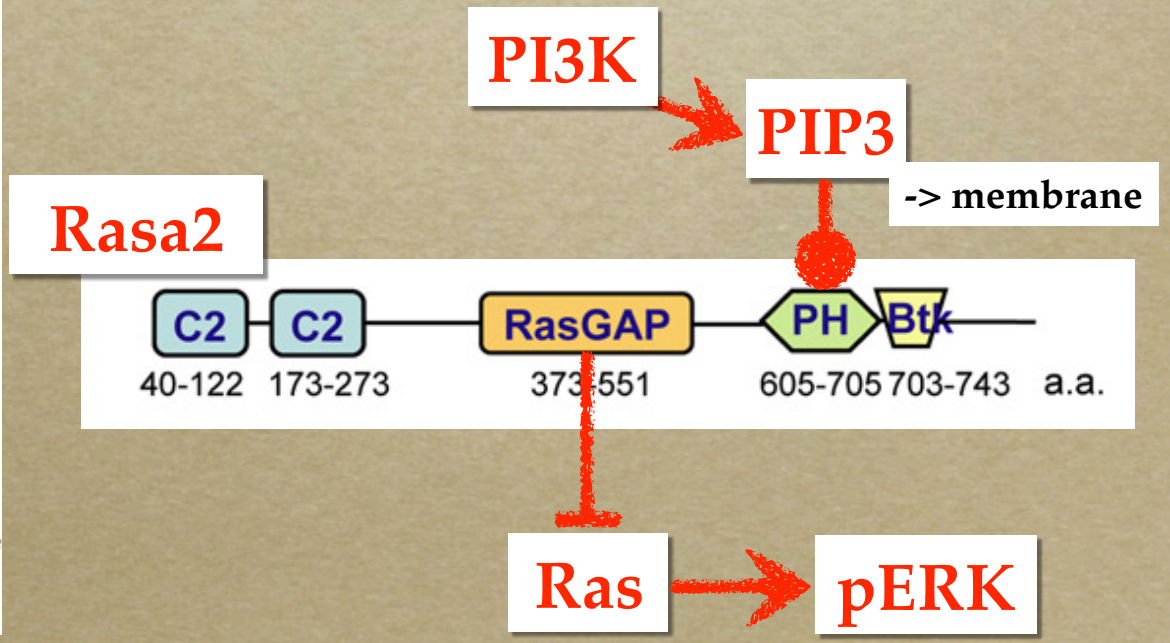
HOW ?

Rasa2 provides a potential mechanism of PI3K induced pERK inhibition

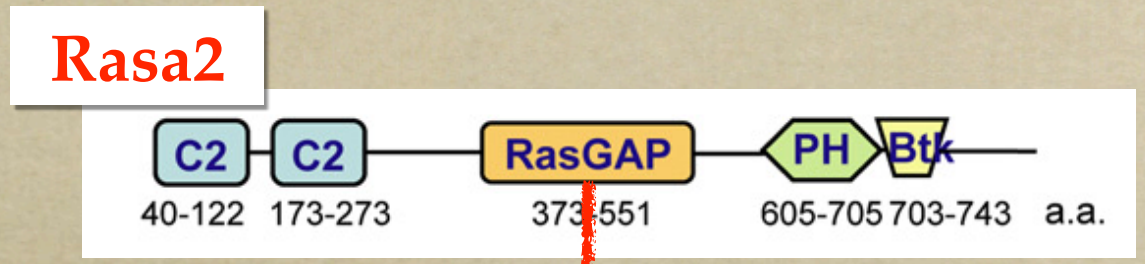


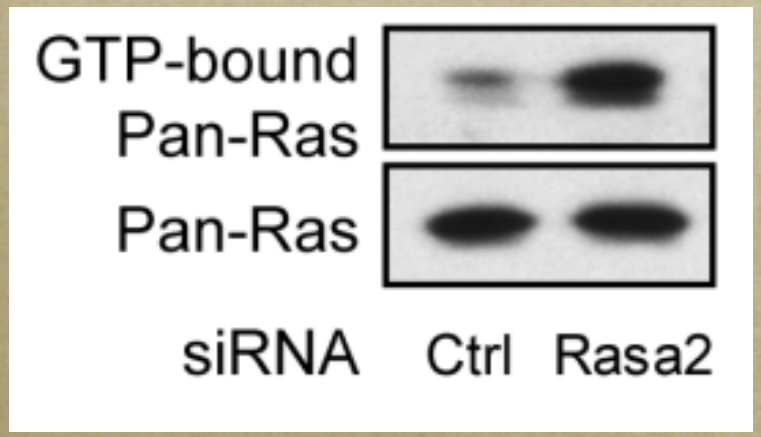




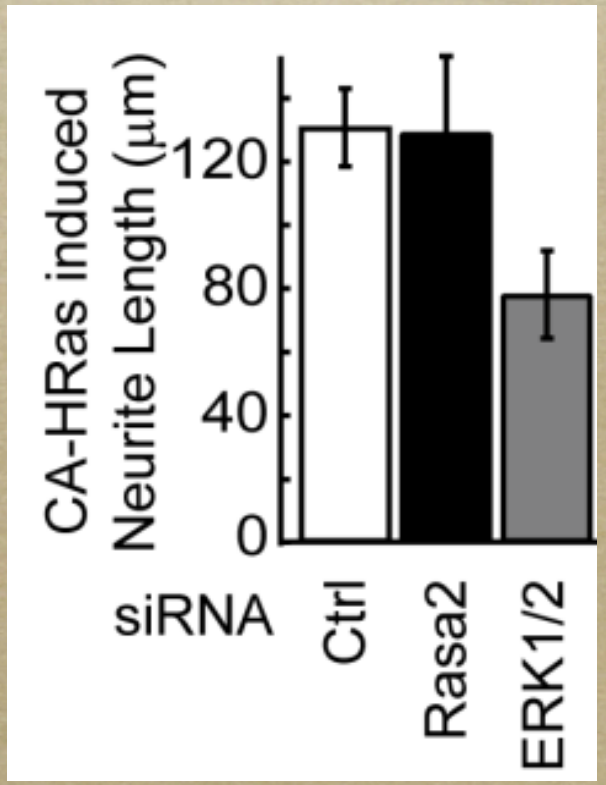


Rasa2 blocks the activity of Ras

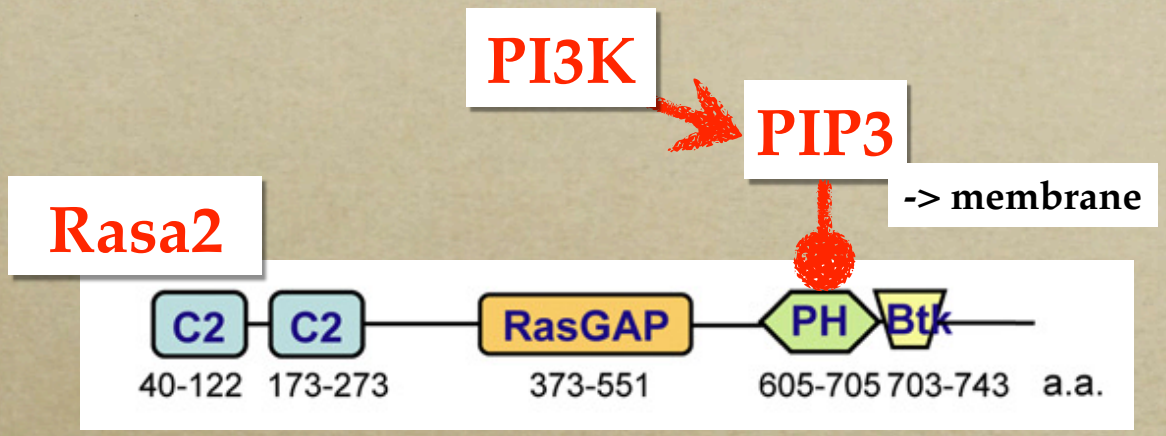


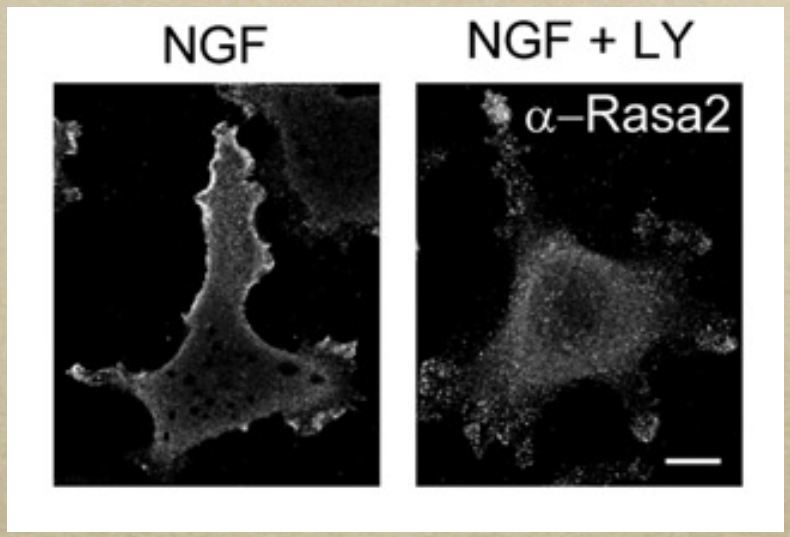


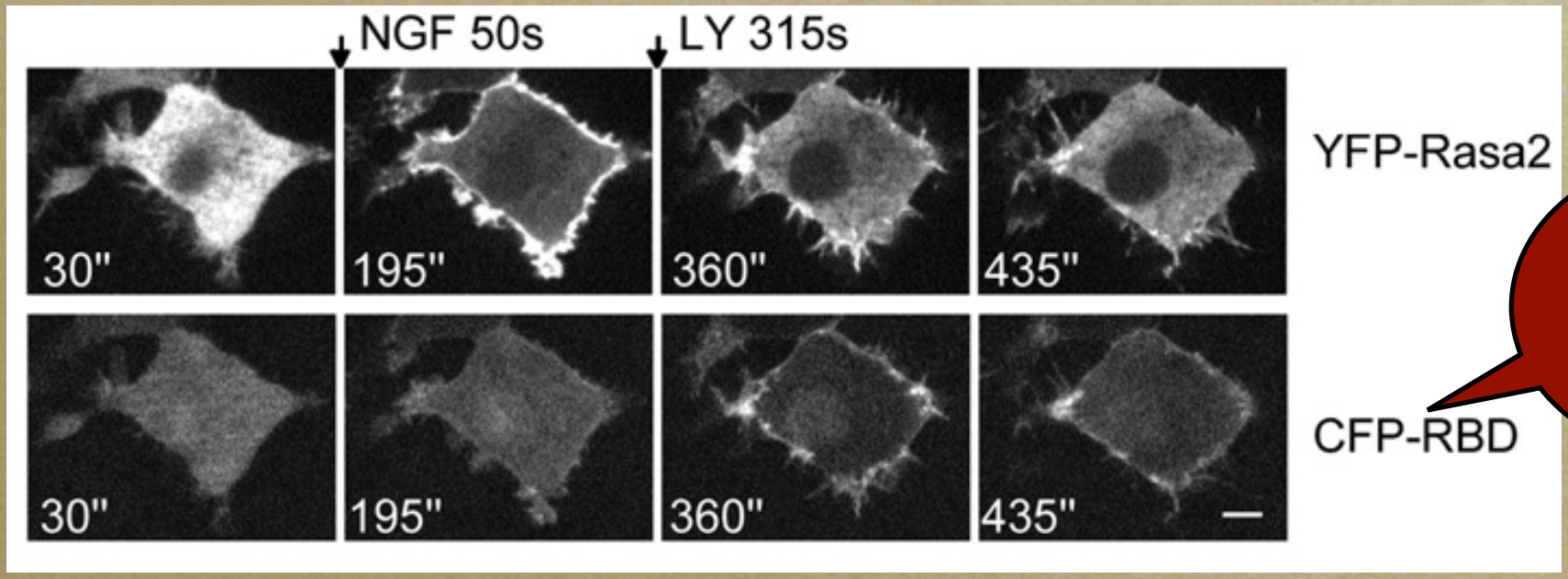
Ras → pERK



Rasa2 membrane localization and RasGAP activity requires active PI3K

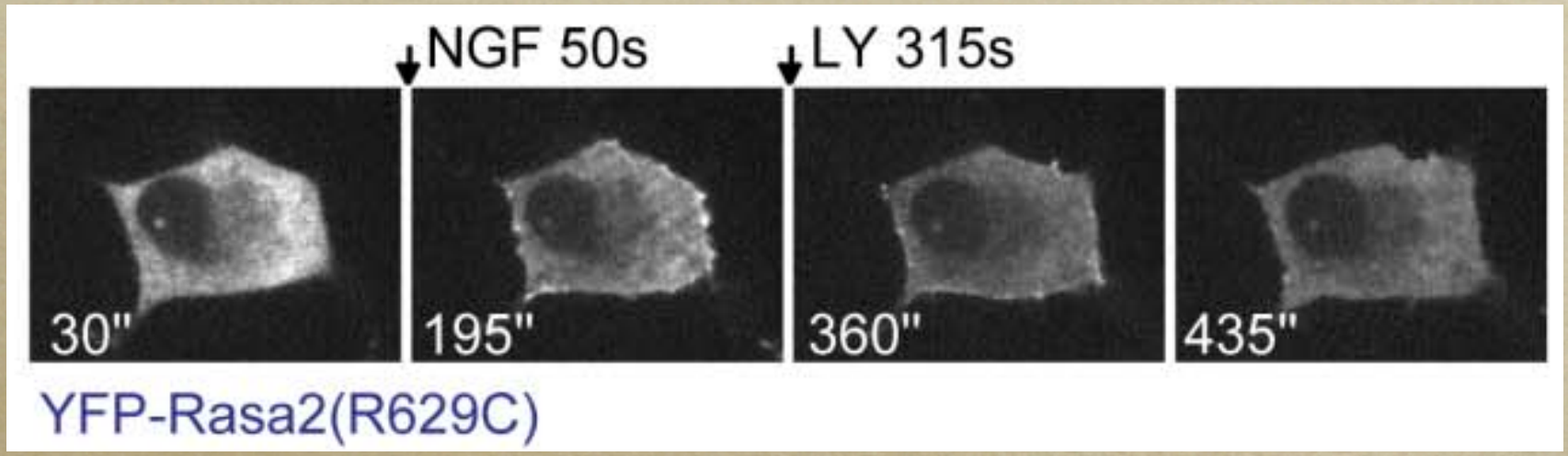


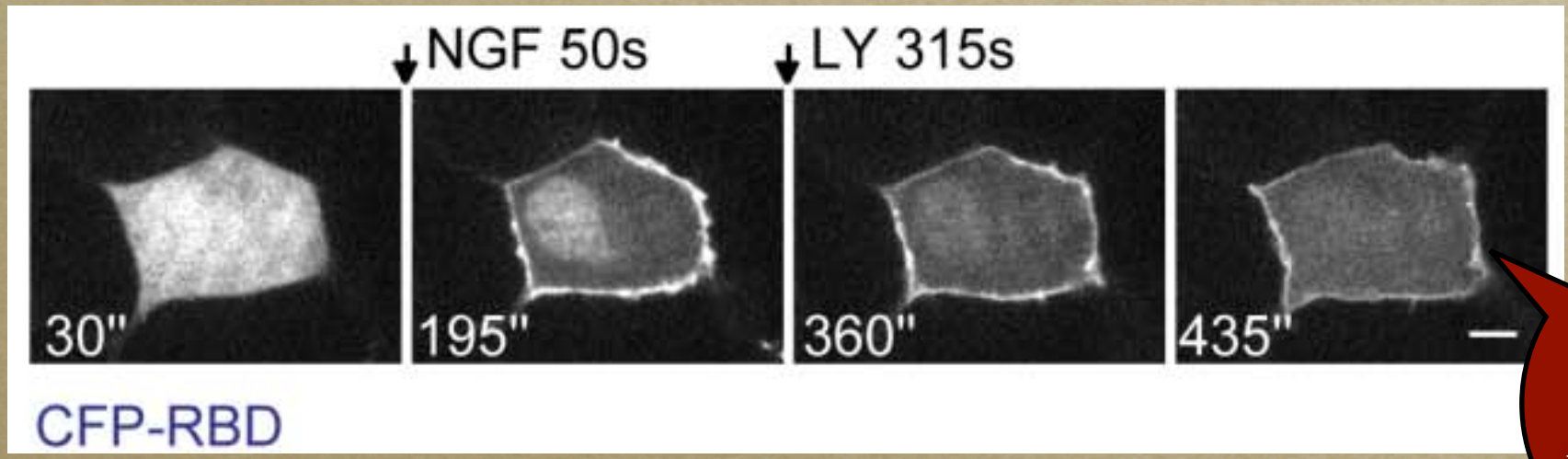




PIP3 binding is critical for RasGAP activity of Rasa2

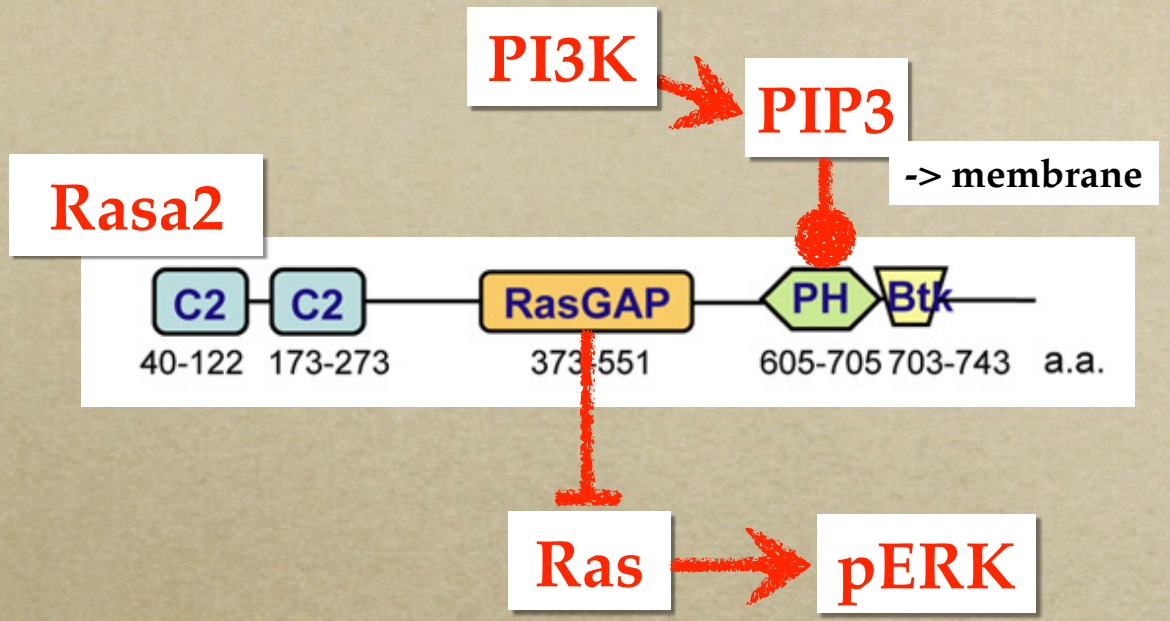
Mutant Rasa2, no PIP3 binding

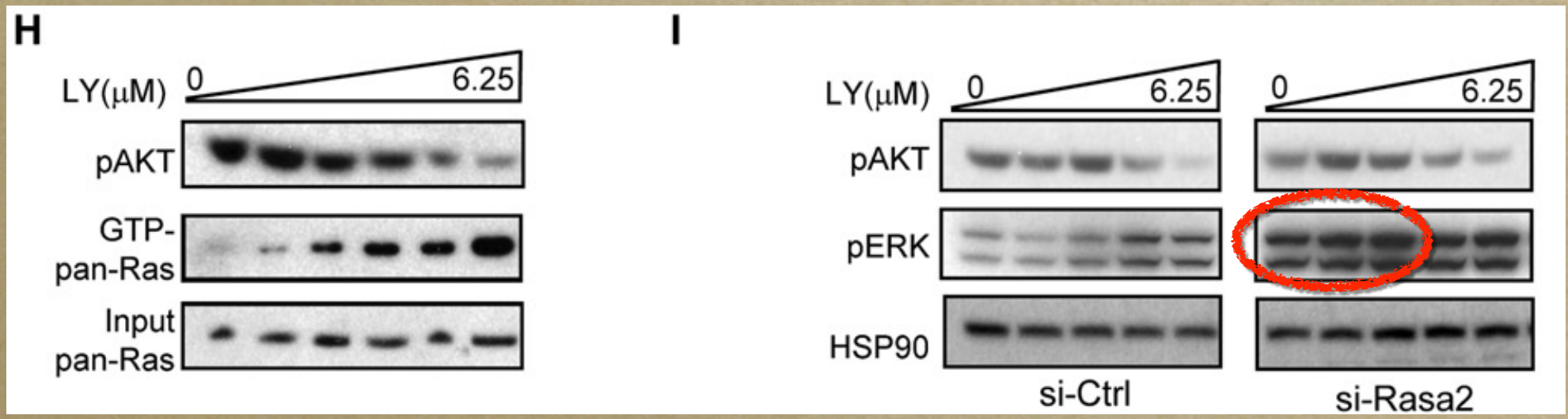




Active RAS biosensor

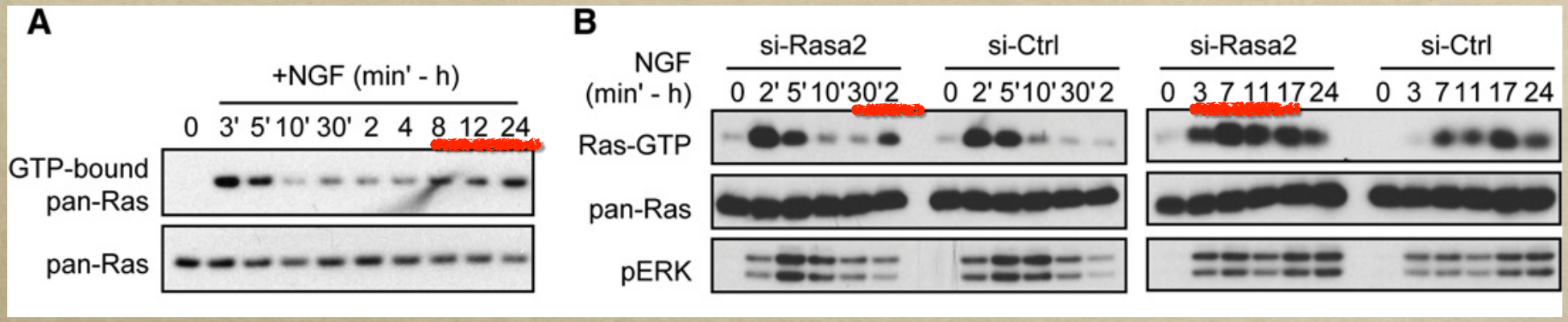
All together (Take-home V.):



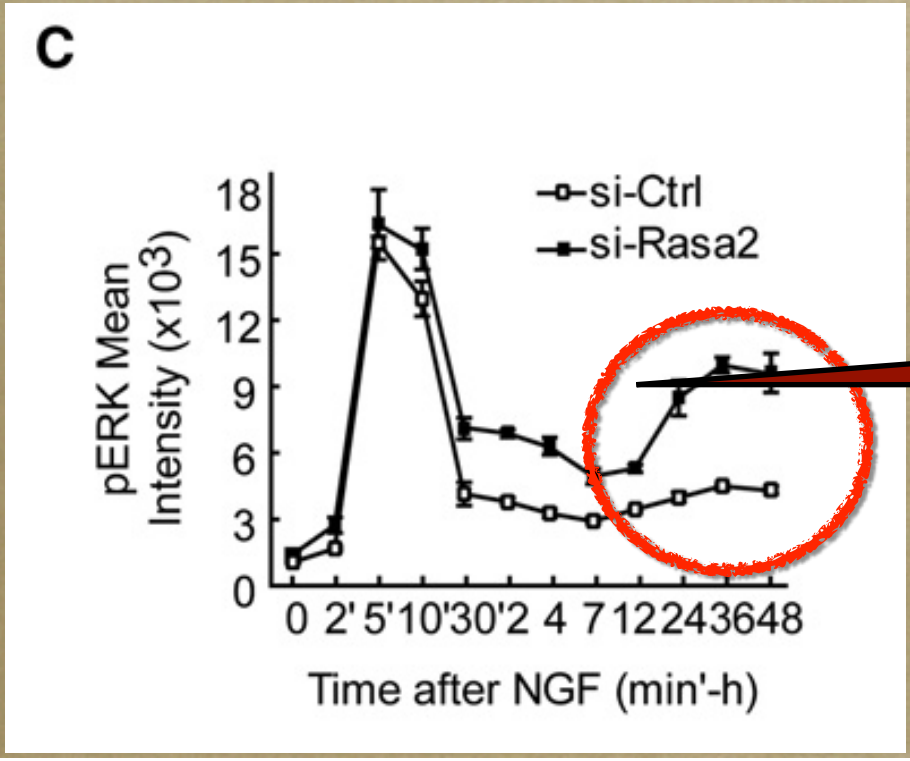


When does Rasa2 regulate cell fate?

NGF induces two waves of RAS activity

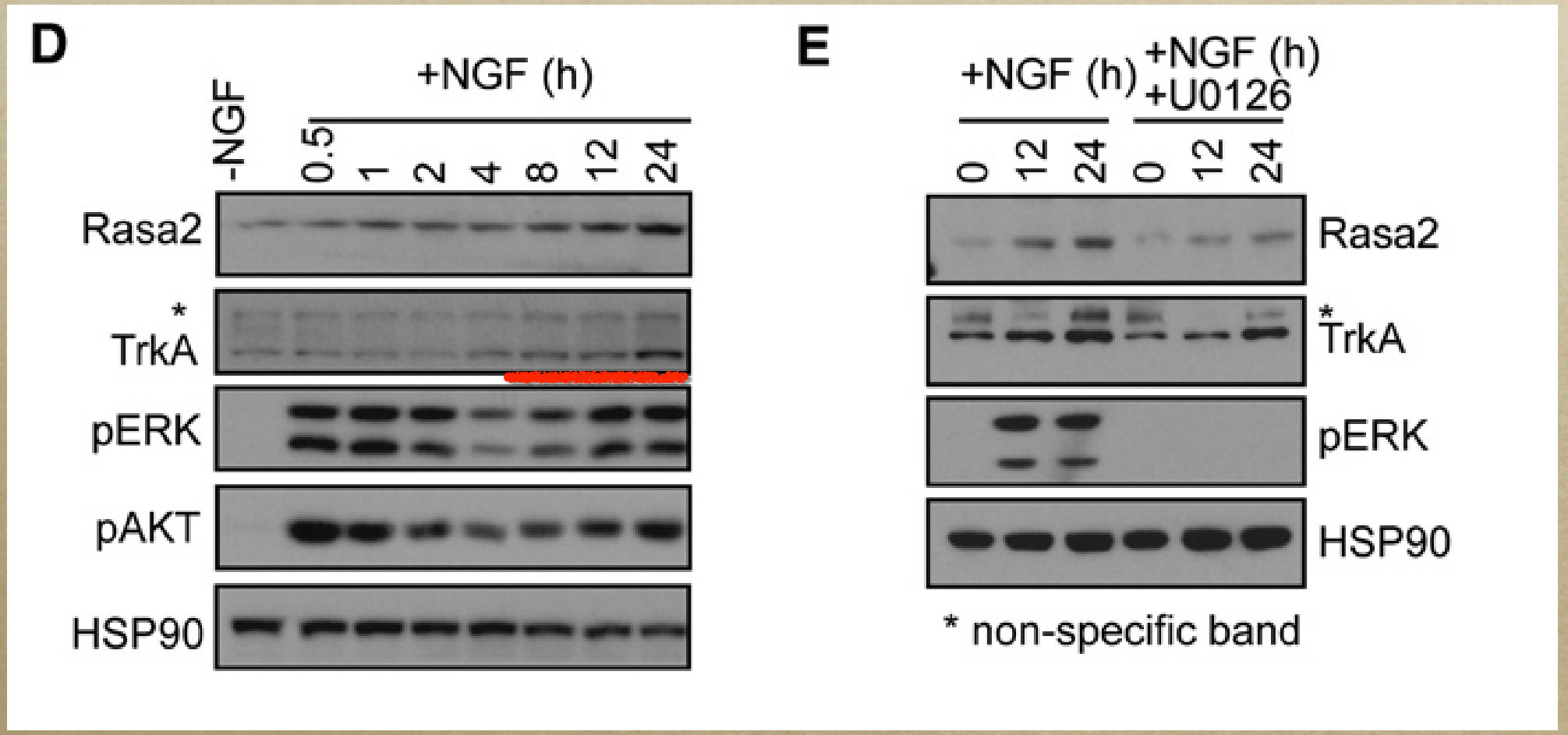


Rasa2 affects the second wave

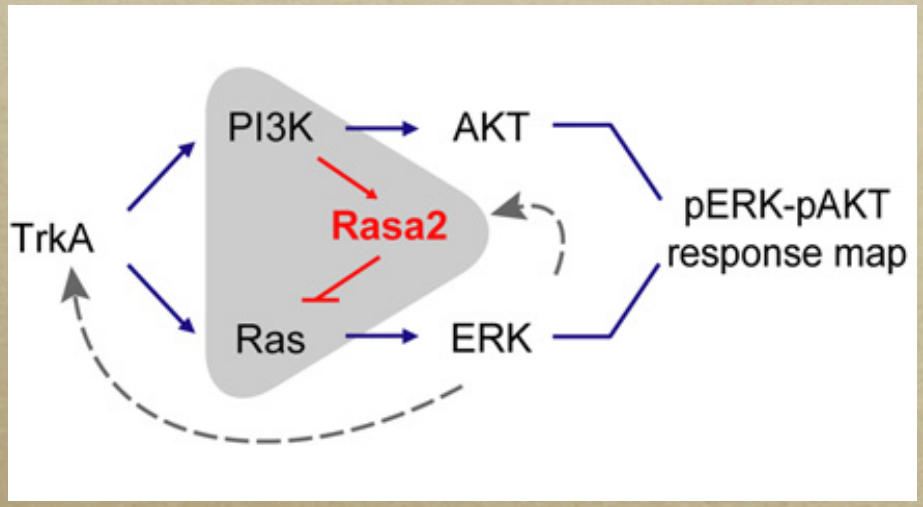


Time of cell fate decision

Second wave: NGF induces expression of its receptor, TrkA, via pERK

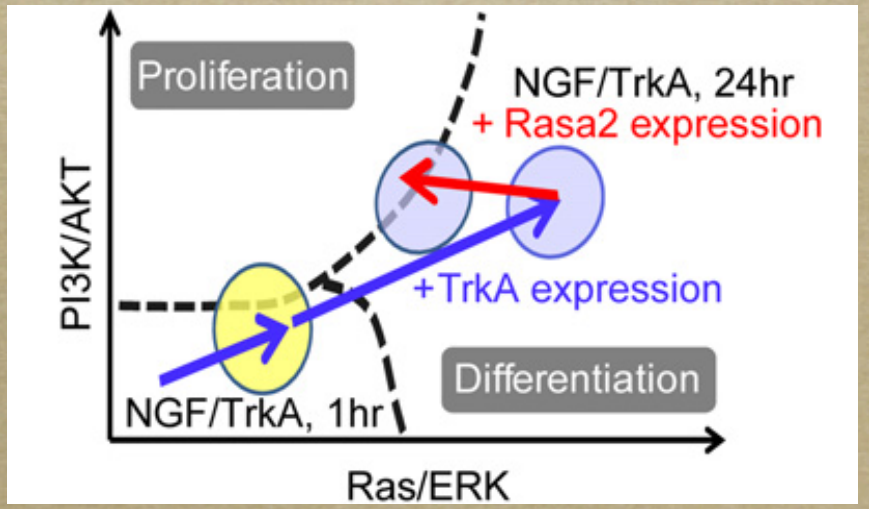


Take-home VI.

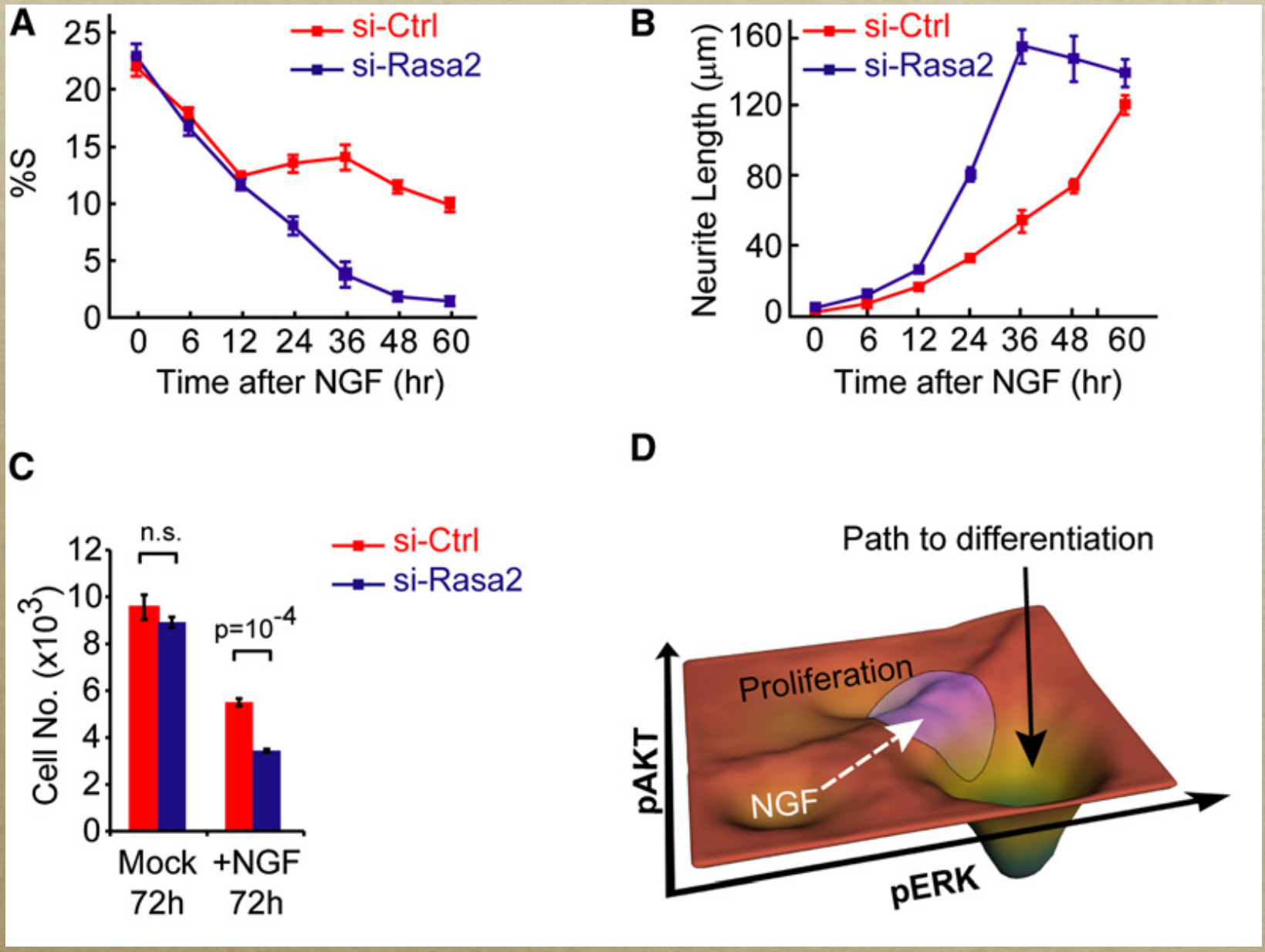


Rasa2 acts as negative feedback on the NGF -> Ras -> pERK -> NGF loop

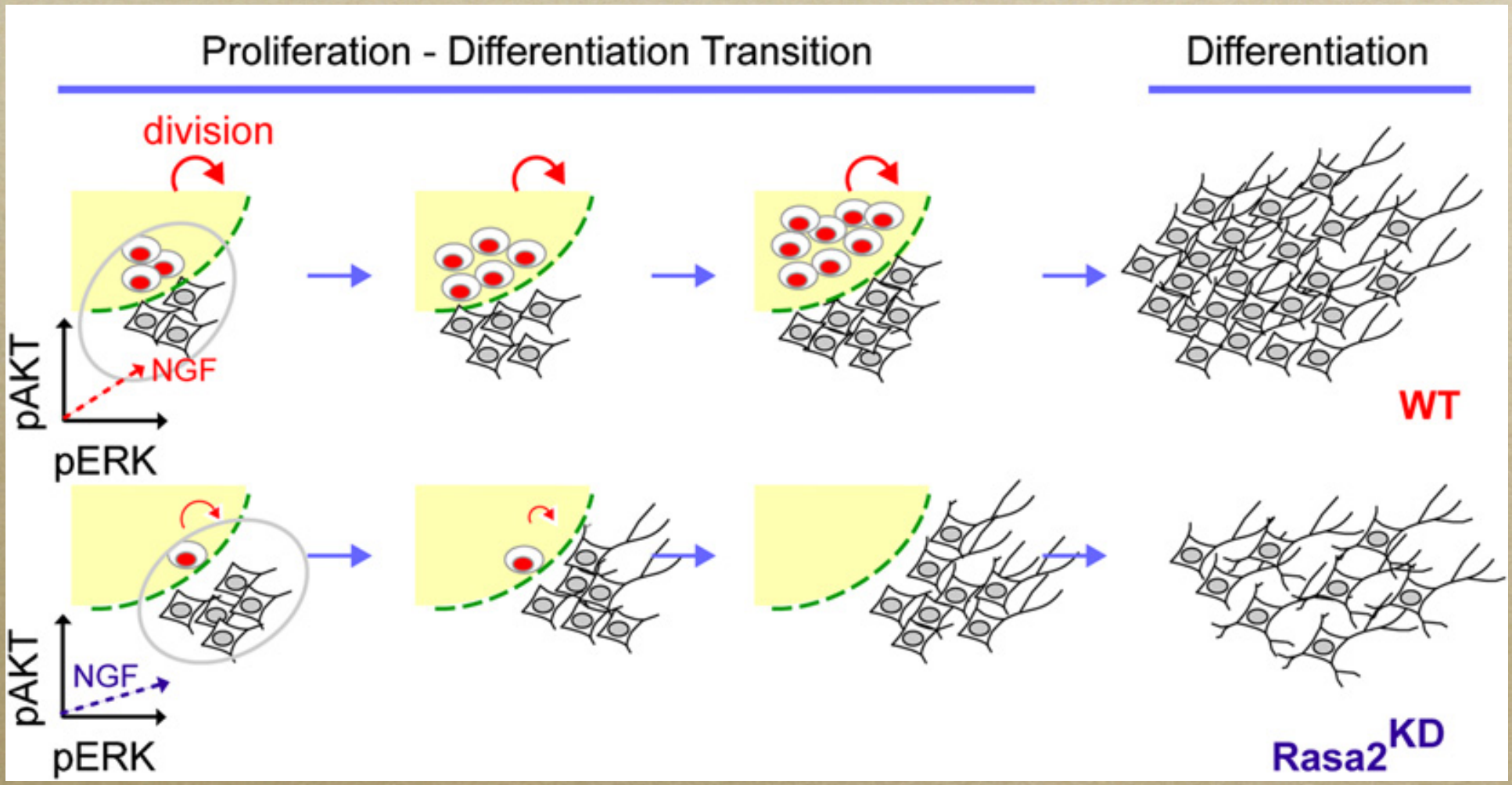
Rasa2 positions the population onto the boundary



Rasa2 helps expand the number of cells during differentiation



Take-home VII.



PC12 cells hedge their bets to perform two mutually exclusive functions - as a population

Conclusions and discussion

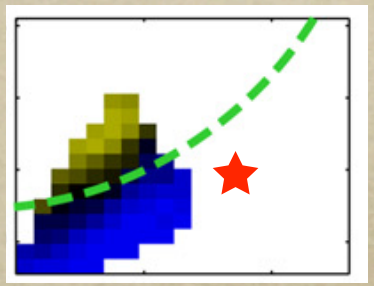
~~Sustained pERK~~

~~Probability of differentiation~~

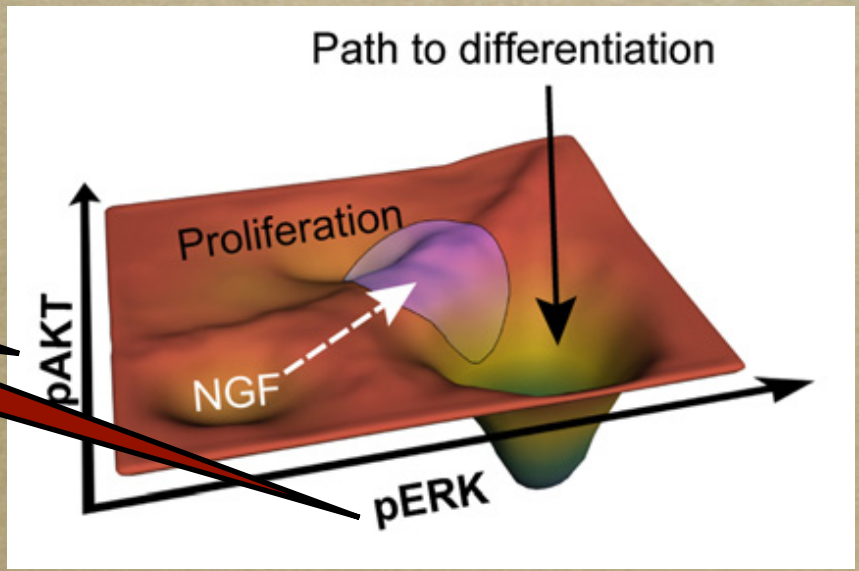
Any point of 2D map

Distance from boundary

Probability of proliferation vs differentiation



Signaling hubs that integrate information

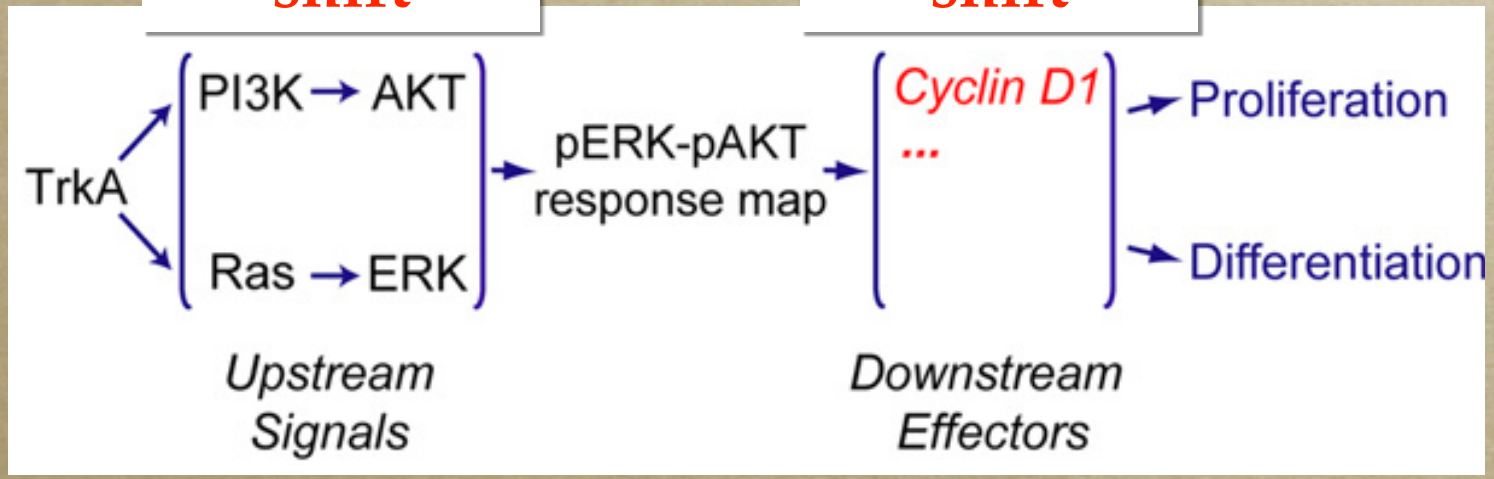


Conclusions and discussion (cont.)

Boundary independent of activating signal

Population shift

Boundary shift



pAKT →

pERK →

CyclinD1/D3 are essential for translating pAKT-pERK map to cell fate

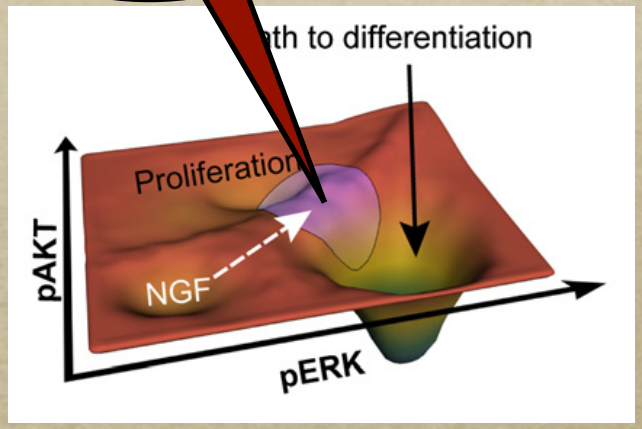
Conclusions and discussion

Bet hedging upon NGF stimulation

Single-cell intrinsic noise

~30% variability in protein expression

Boundary sharper than signal variation



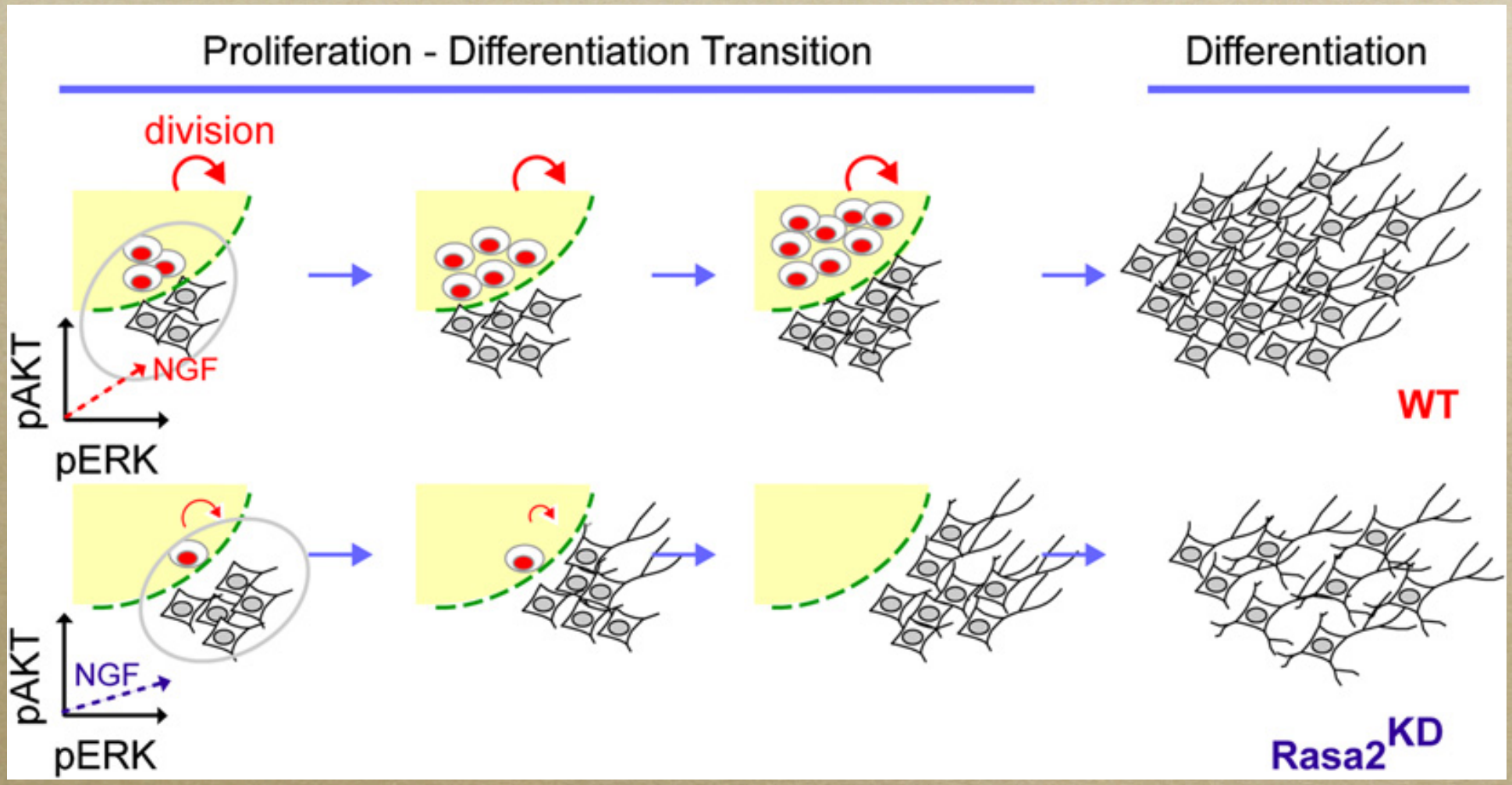
Mechanisms of dampening noise

Robust signal processing

Mechanisms of harnessing noise

Cellular decision making & bet hedging

Bet hedging relies on Rasa2 feedback to position population onto the boundary



As a population, PC12 cells perform two mutually exclusive functions

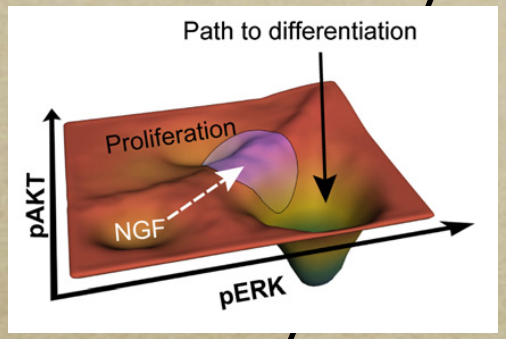
Summary

Single-cell intrinsic noise

~30% variability in protein expression

Control to harness noise

Cellular decision making & bet hedging



1. Response map with sharp boundary
2. Enough signal variation to spread signal across boundary
3. Negative feedback to position population near boundary

Tight control over population percentages

Strengths

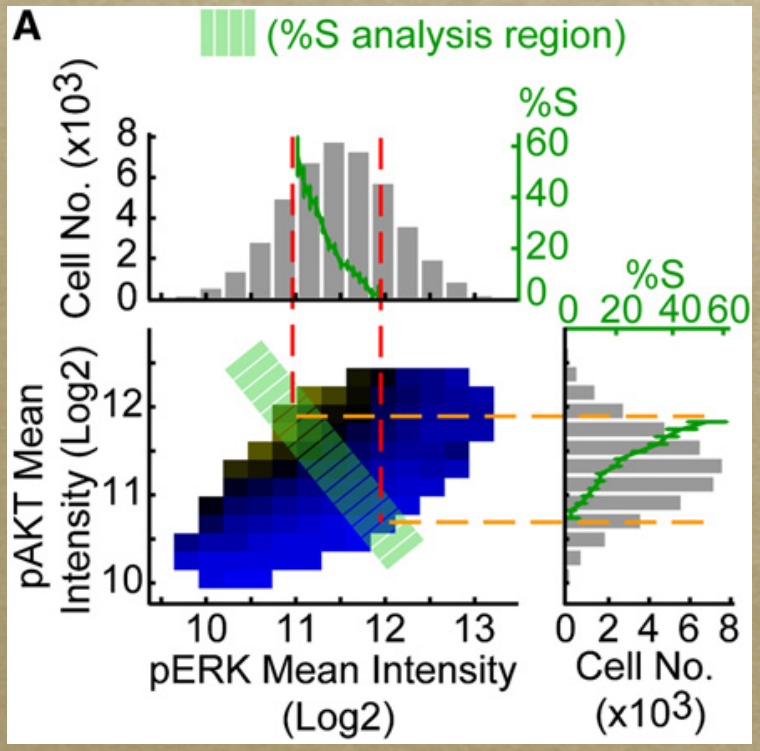
Strengths:

- Conceptual backbone -> insight into how the signaling is structured
- Very clear logic, beautiful flow
- 2 hits in 1308 siRNA screen with large consistent effect -> both tied to pERK/pAKT system with direct interactions

Strengths and weaknesses (cont.)

Weaknesses:

- Experimental? (still not quite qualified to really know…)
- Conceptual (small weakness, in discussion)



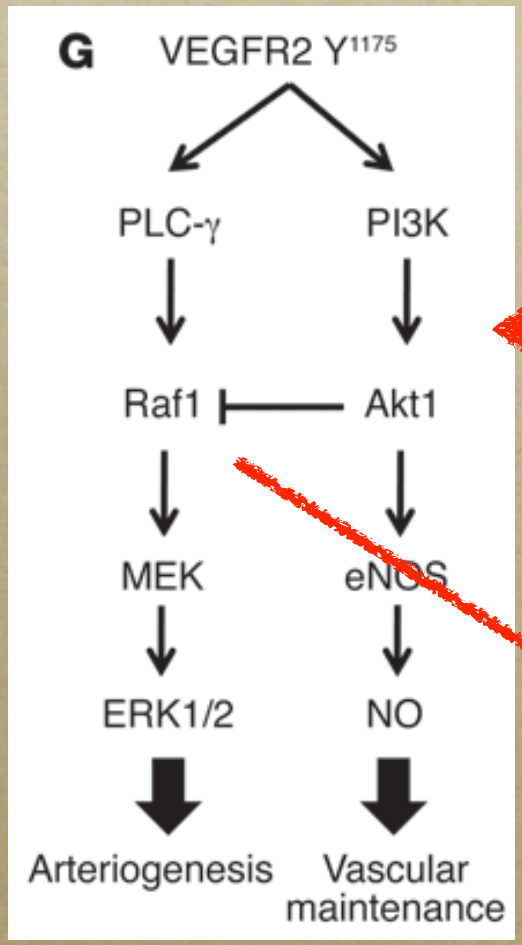
Response in pAKT & pERK is perfectly unimodal

NEED multi-stable switches downstream

Not discussed at all…

Outlook: AKT and ERK in endothelial biology

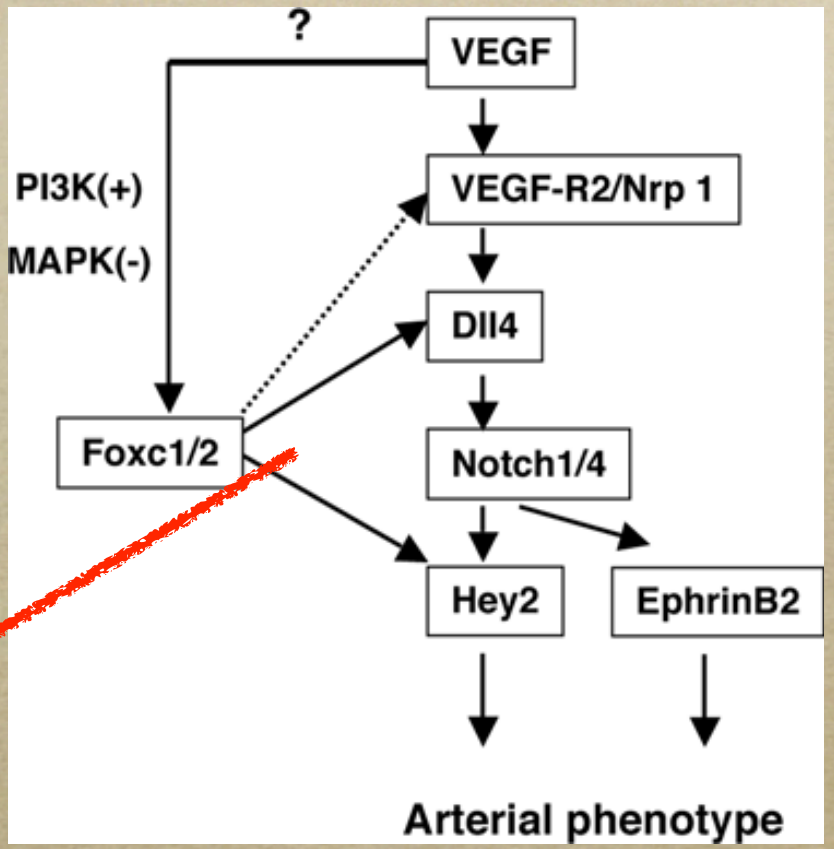
Vascular remodeling



?

?

tip/stalk patterning



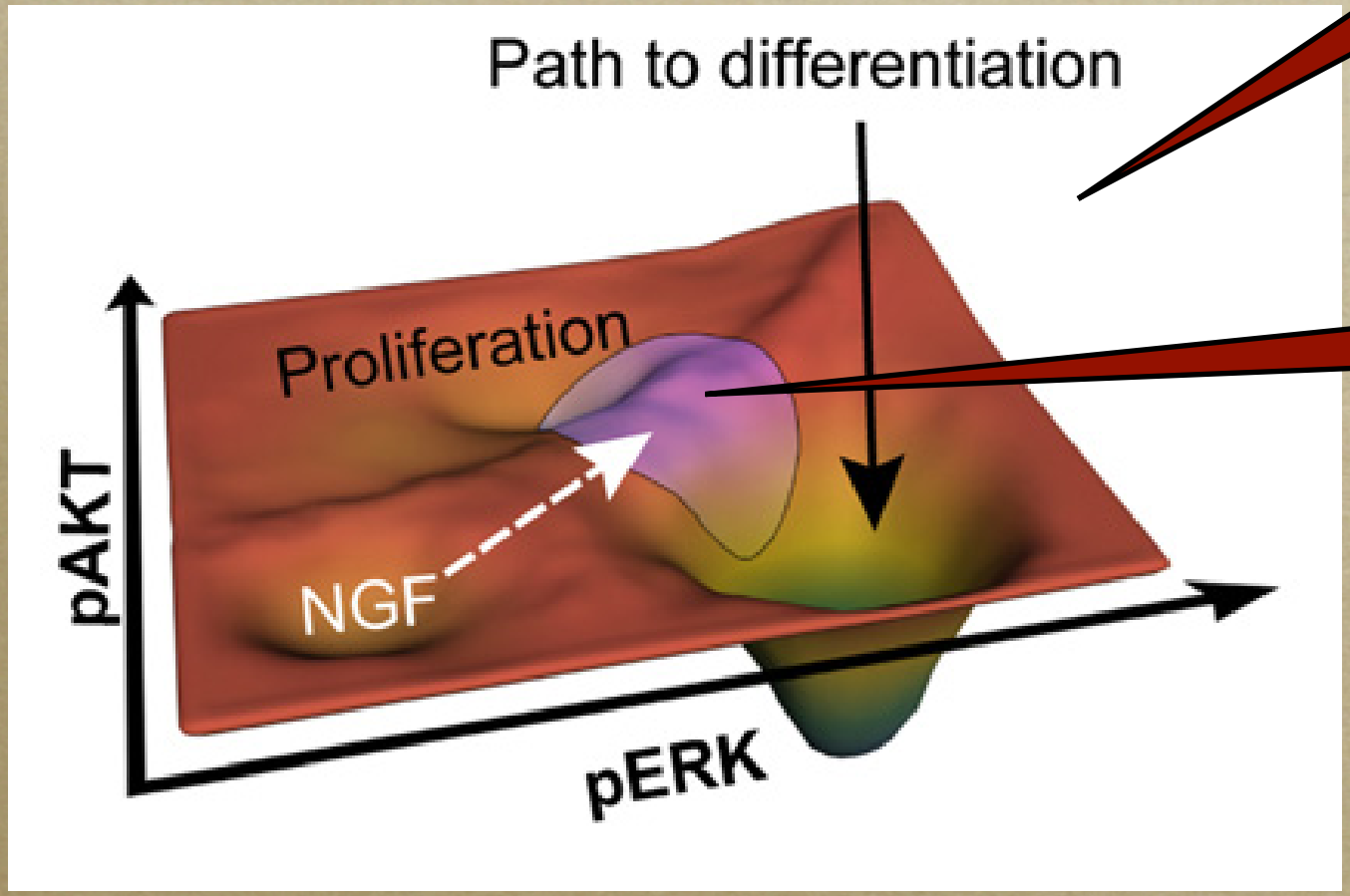
Ren, B. *et al.* ERK1/2-Akt1 crosstalk regulates arteriogenesis in mice and zebrafish. *J Clin Invest* 120, 1217–1228 (2010).

Hayashi, H. & Kume, T. Foxc transcription factors directly regulate Dll4 and Hey2 expression by interacting with the VEGF-Notch signaling pathways in endothelial cells. *PLoS ONE* 3, e2401 (2008).

Food for thought

What does this 2D map look like in ECs?

How does the boundary depend on context?



input to EC
angiogenic sprouting
arterial fate specification
arterial fate maintenance
inflammation