# Activation of PI3K from RAS


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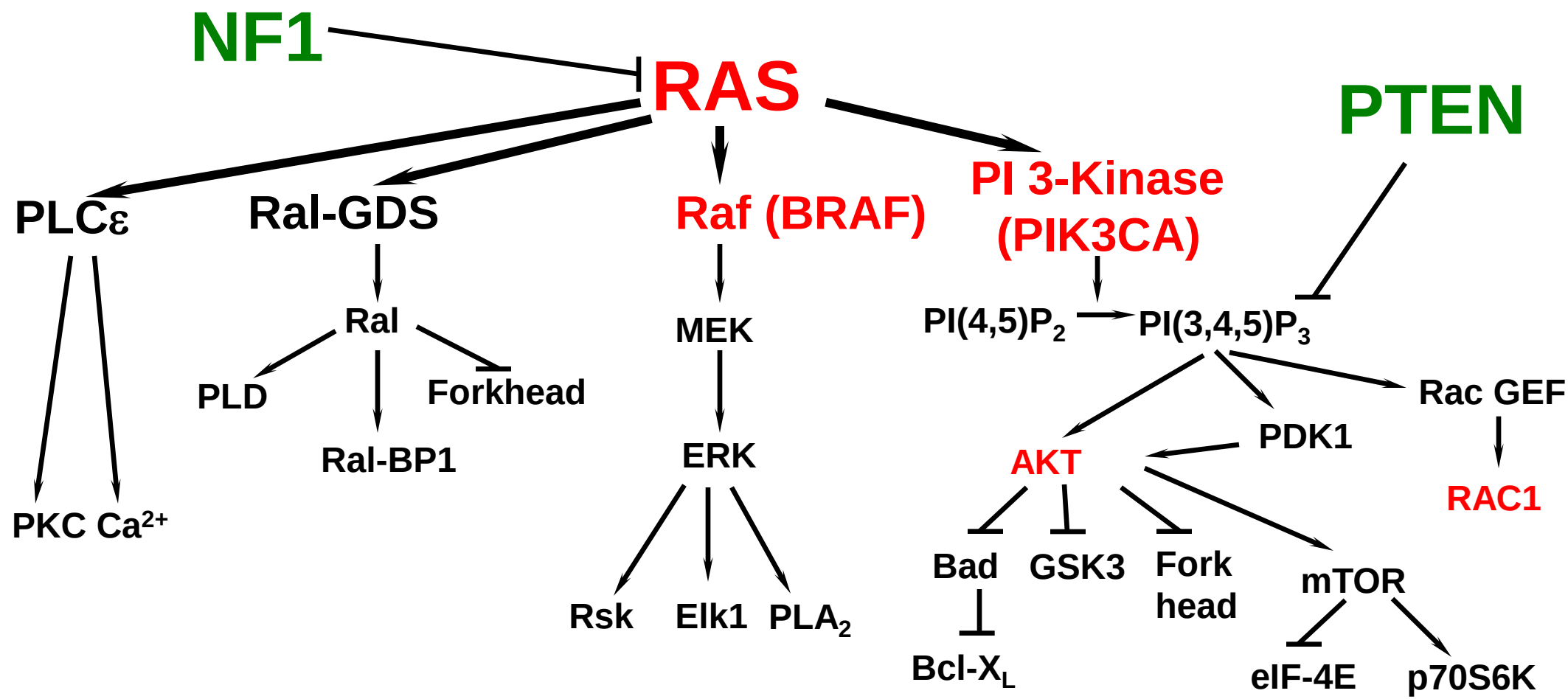
**ESMO Symposium on Signalling Pathways**

**Barcelona**

**Spain**

**13th March 2015**

# The major RAS signalling pathways




Calcium signalling

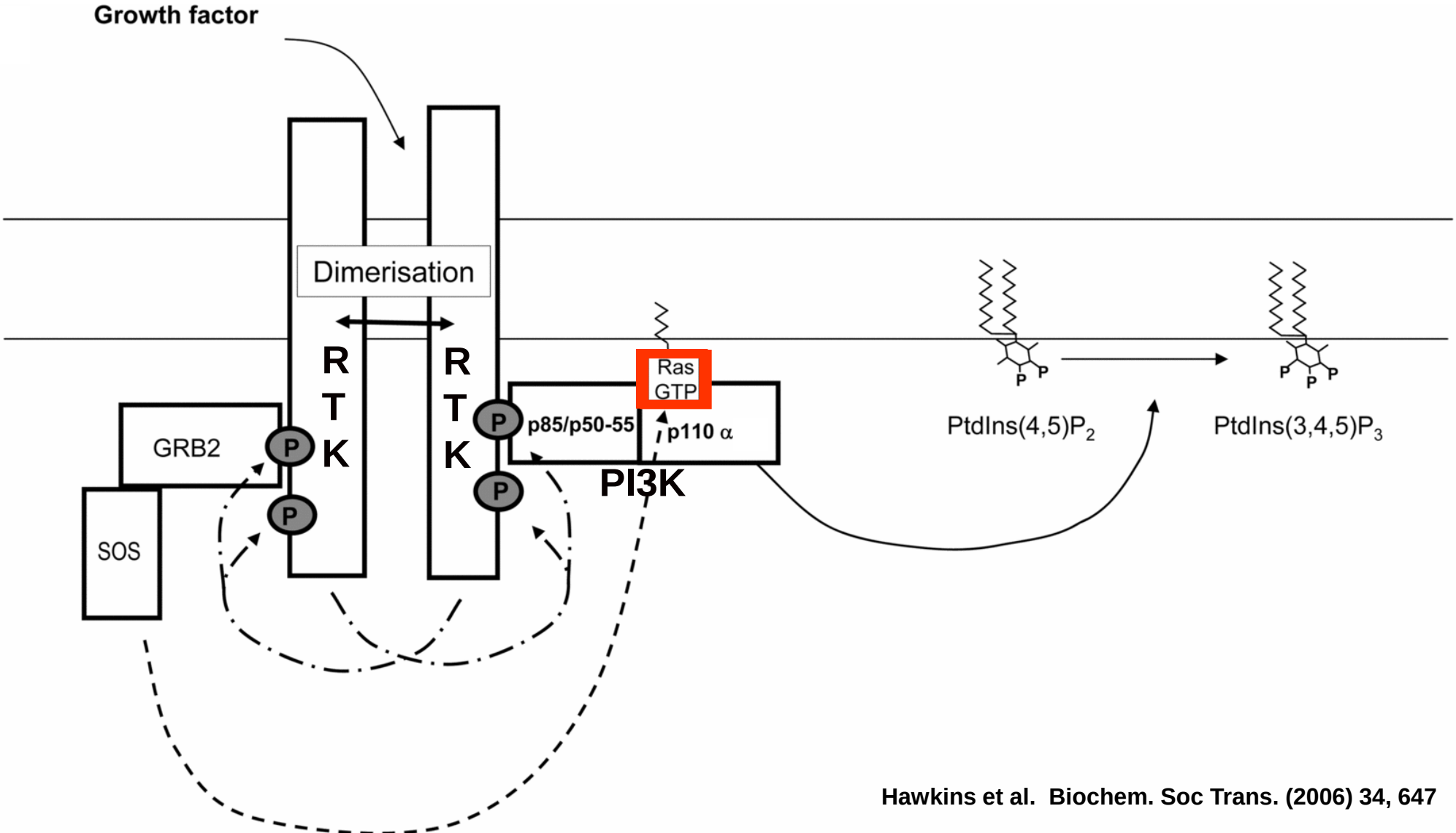
Proliferation
Transcription
Endocytosis

Proliferation
Transcription

Survival
Proliferation
Transcription

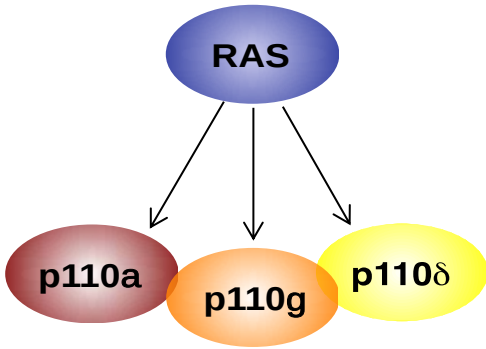
Translation
Cytoskeleton

# Activation of PI 3-kinase by growth factors involves interaction of PI3K regulatory subunit with receptor tyrosine kinase and PI3K catalytic subunit with RAS

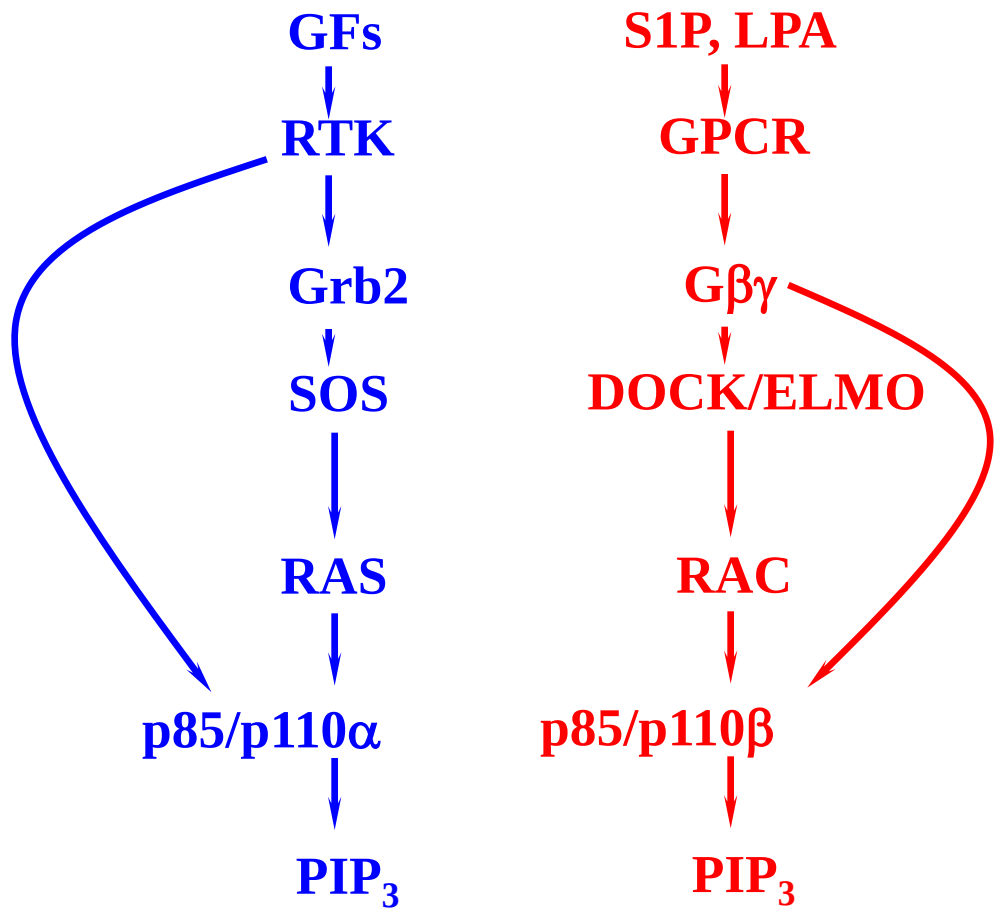


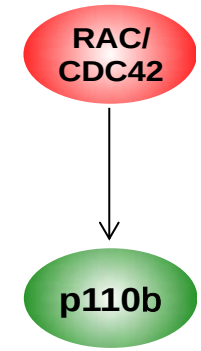

Hawkins et al. Biochem. Soc Trans. (2006) 34, 647

# RAS binds p110α, γ and δ, RAC binds p110β




RAS binders




RHO binder

*R. Fritsch, I. de Krijger, K. Fritsch, R. George, B. Reason, M.S. Kumar, M. Diefenbacher, G. Stamp, J. Downward (2013) Cell 153, 1050-1063. "RAS and RHO families of GTPases directly regulate distinct phosphoinositide 3-kinase isoforms."*

# Possible ways to selectively target RAS mutant cancer cells

## 1. Inhibit RAS function directly (target oncogene addiction)

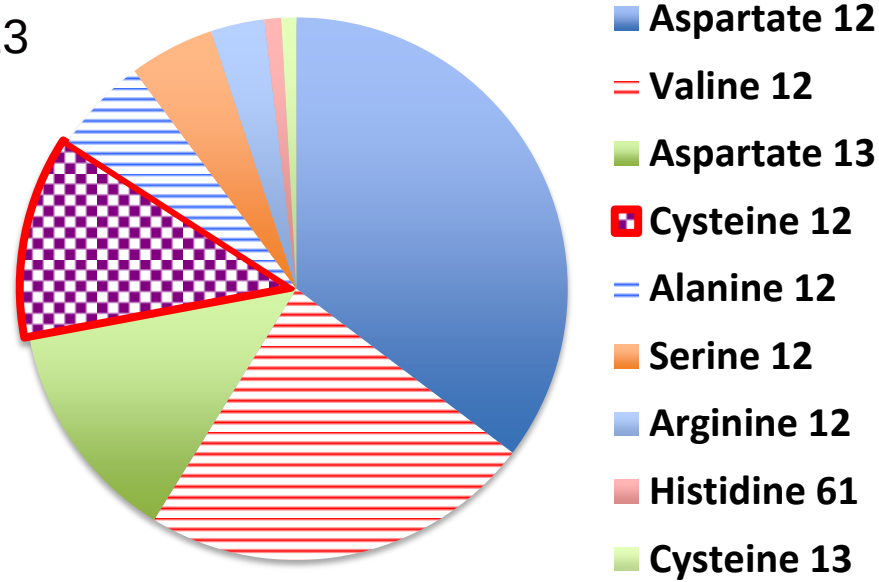
LETTER

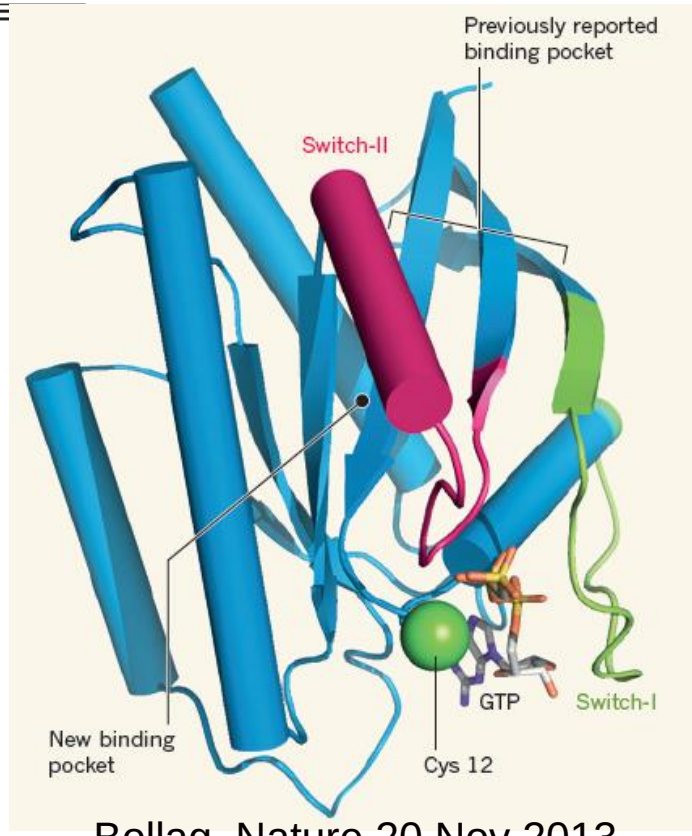
doi:10.1038/nature12796

### K-Ras(G12C) inhibitors allosterically control GTP affinity and effector interactions

Jonathan M. Ostrem[1]*, Ulf Peters[1]*, Martin L. Sos[1], James A. Wells[2] & Kevan M. Shokat[1]

Nature 20 Nov 2013



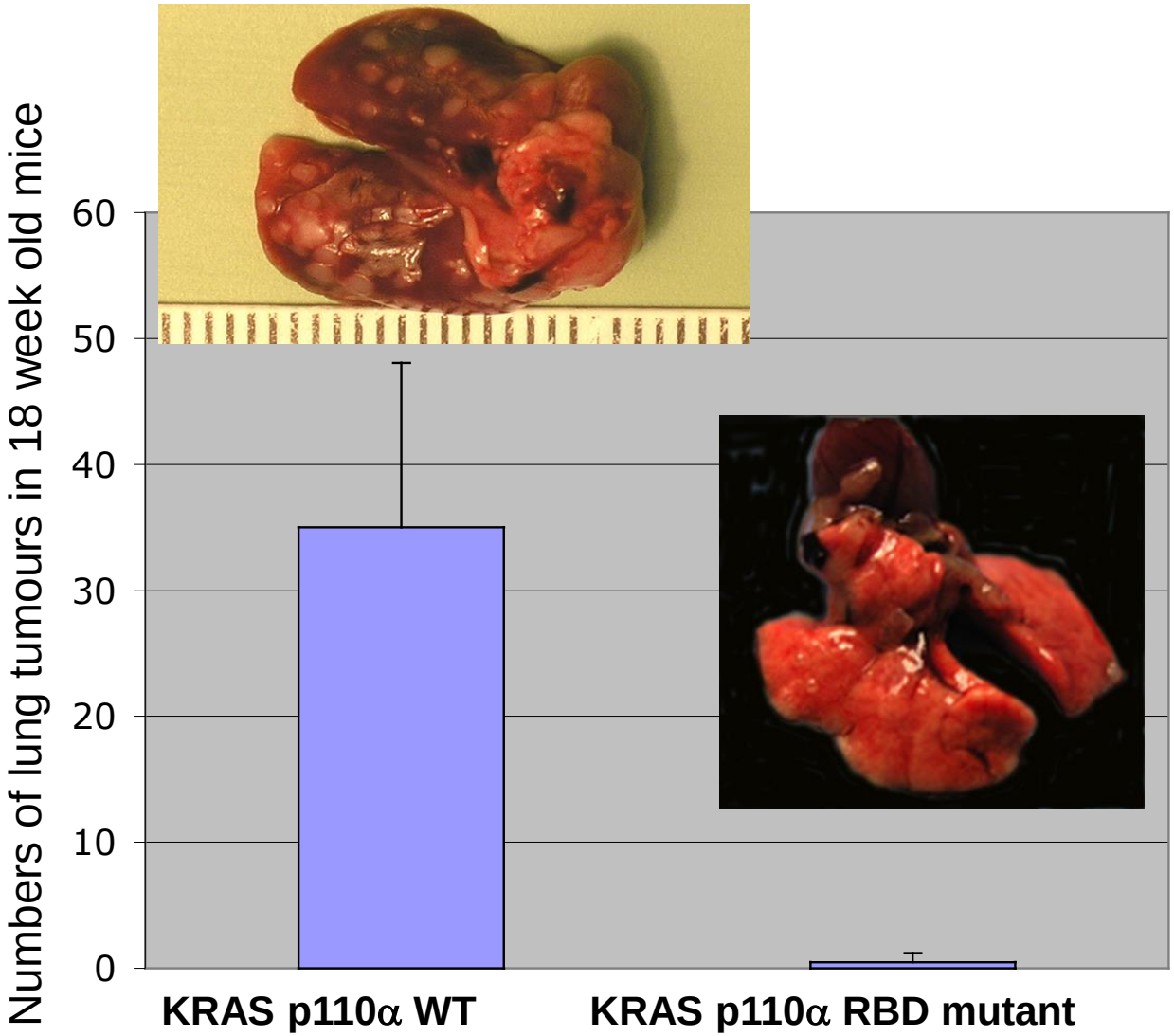




Bollag, Nature 20 Nov 2013

# Possible ways to selectively target RAS mutant cancer cells

1. Inhibit RAS function directly (target oncogene addiction)
2. **Inhibit RAS controlled downstream signaling enzymes, e.g. RAF, PI3K, mTOR (target oncogene addiction indirectly)**

# Mice with mutant RAS interaction site in PI3K p110α show massively reduced KRAS induced lung tumour rates




**KRAS LA2 mice**

**Tony Ramjaun**
**Surbhi Gupta**

*S. Gupta, A.R. Ramjaun, P. Haiko, P.H. Warne, E. Nye, G. Stamp, K. Alitalo and J. Downward (2007) Cell 129, 957-968. "The interaction of Ras with p110α, the catalytic subunit of PI 3-kinase, is required for Ras-driven tumorigenesis and for normal lymphatic development."*

# Is the interaction of RAS with PI3Kα required for tumour maintenance as well as formation?

PIK3CA-RBD/ PIK3CA-loxP
Rosa26 Cre-ER / wt
K-RasG12D LA2/ wt

PIK3CA-wt/ PIK3CA-loxP
Rosa26 Cre-ER / wt
K-RasG12D LA2 / wt

PIK3CA-wt/ PIK3CA-wt
Rosa26 Cre-ER / wt
K-RasG12D LA2 / wt

Age to 2-3 months to allow latent KRAS G12D driven lung tumorigenesis



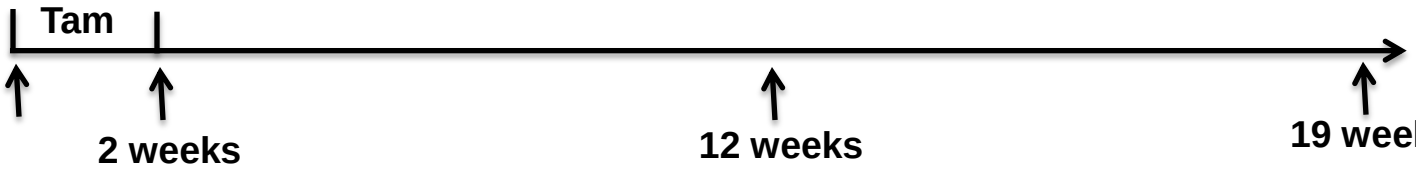
Feed with tamoxifen pellets for two weeks to activate Cre deletion of PIK3CA-loxP



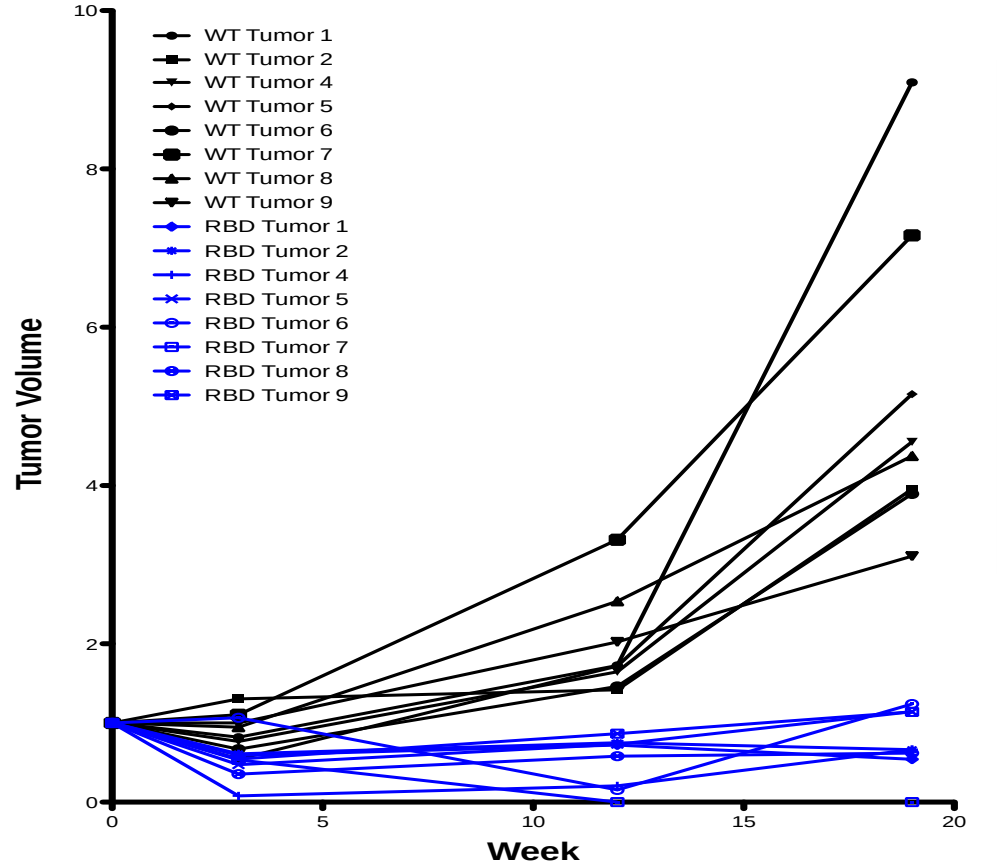
Assessment of lung tumor burden at age 4-5 months

Esther Castellano
Clare Sheridan

# Effect of inducible p110α-RBD mutant expression on size of established KRAS LA2 lung tumours (CT scans)




*E.Castellano, C.Sheridan, ... J.Downward (2013)* ***Cancer Cell****, 24, 617*

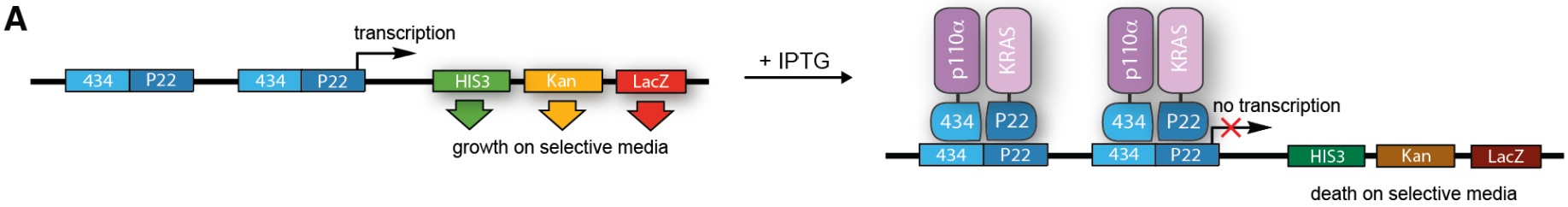


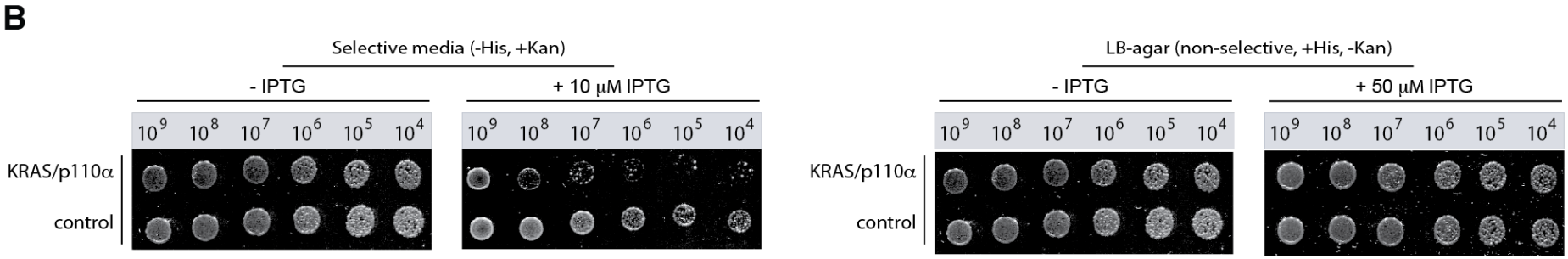

**WT p110α**

**RBD mutant p110α**

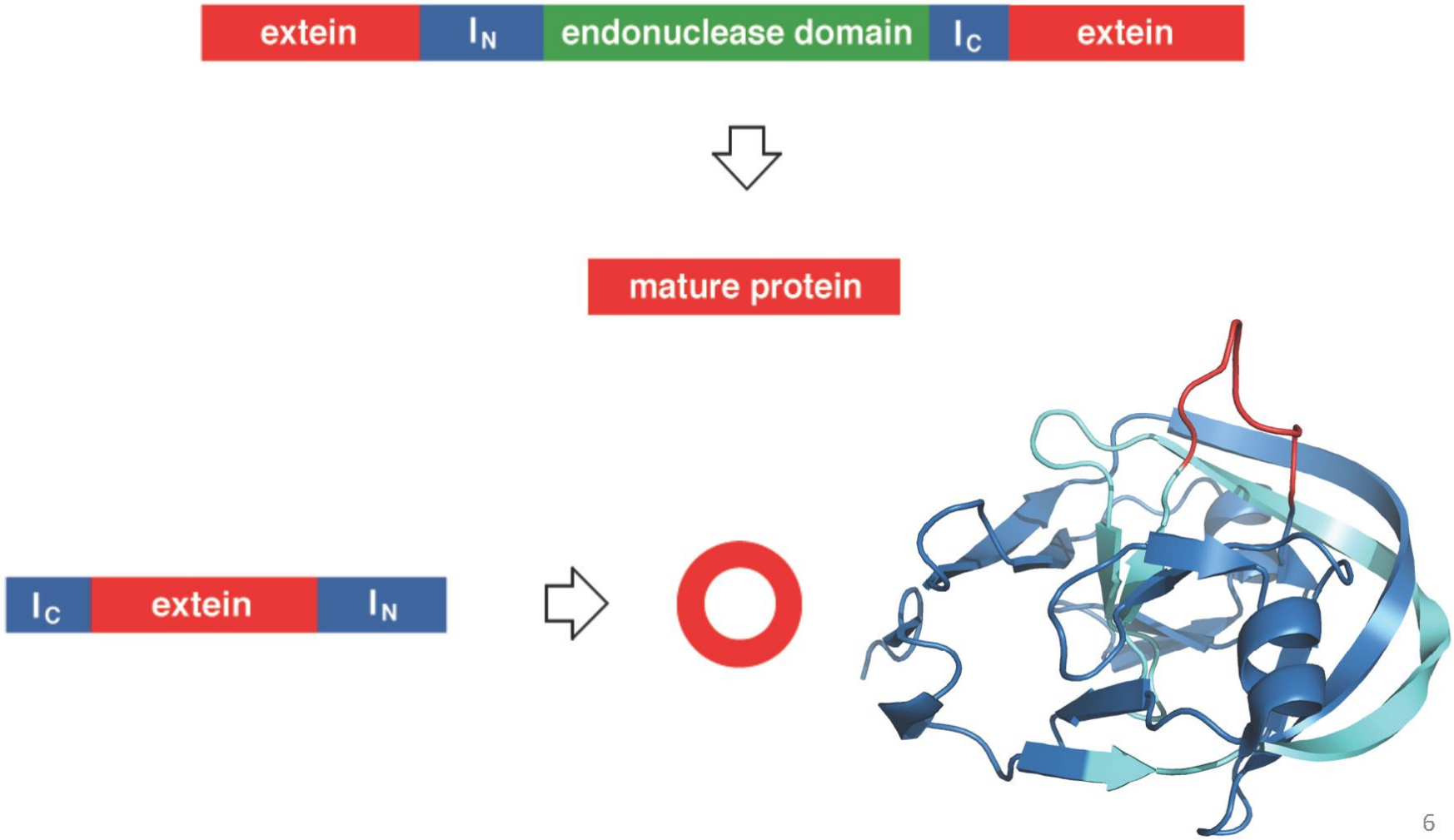
**Removal of KRAS – p110α interaction in existing lung tumours leads to long term tumour stasis and partial regression**

# A genetically encoded platform for the identification of protein-protein interaction inhibitors.

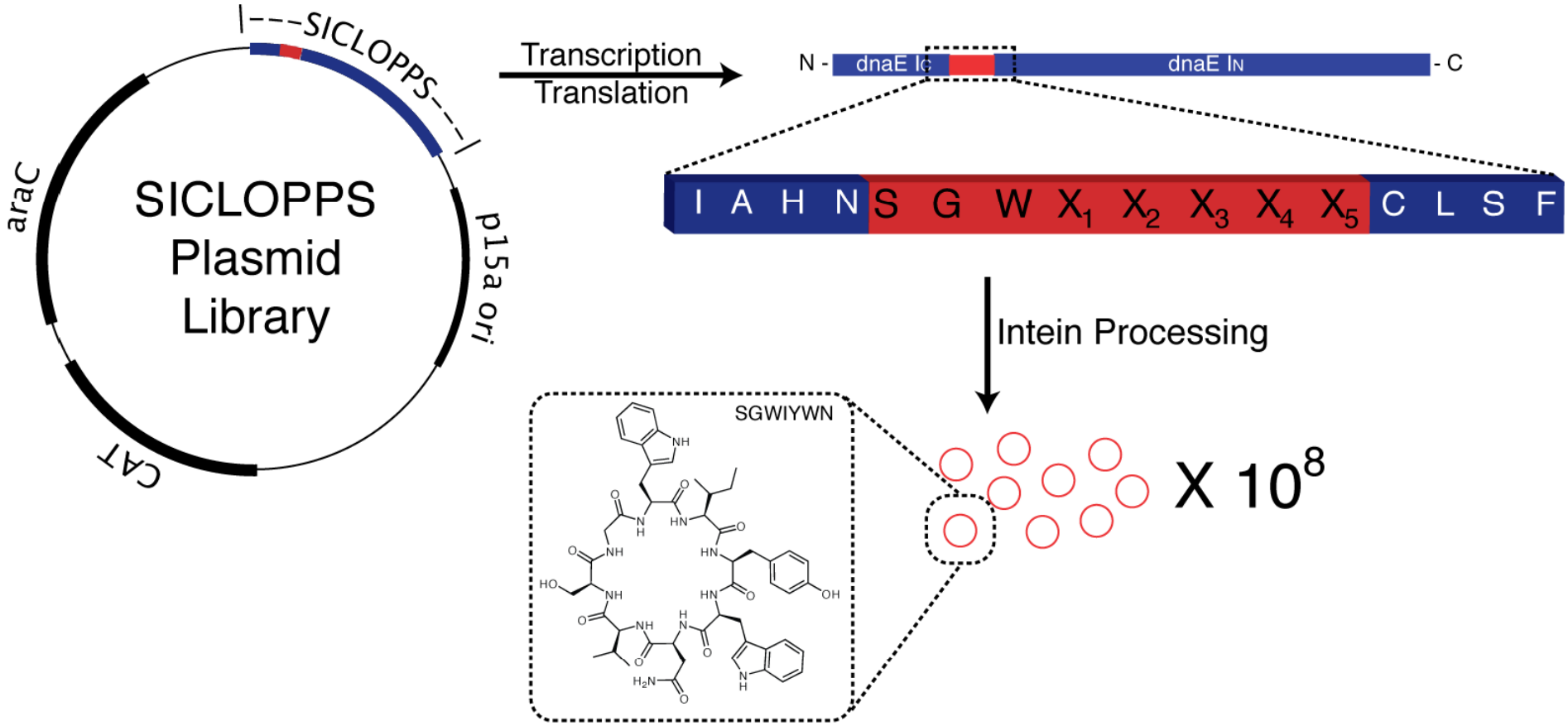


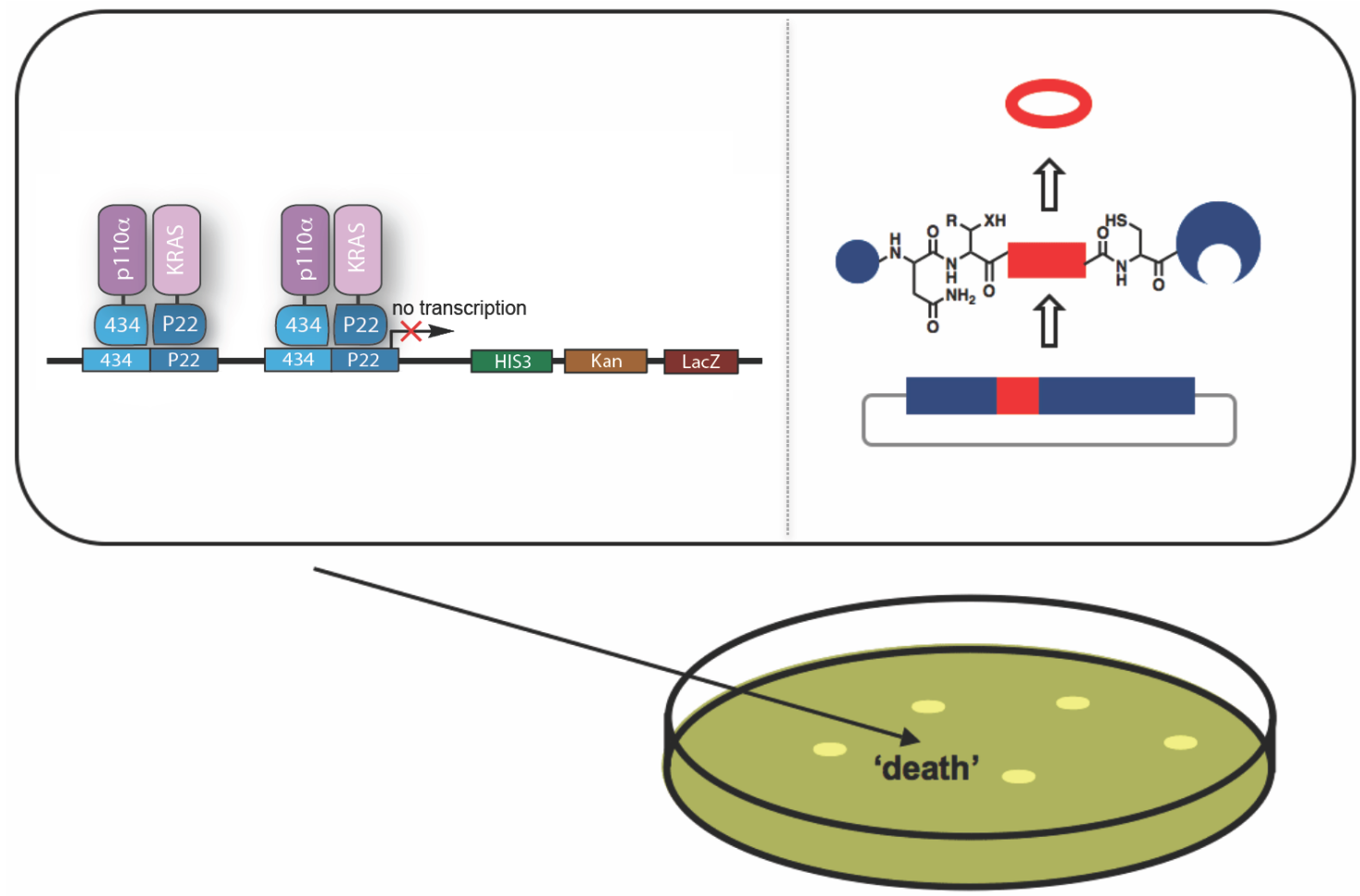
# Split-Intein Circular Ligation of Proteins and Peptides: SICLOPPS



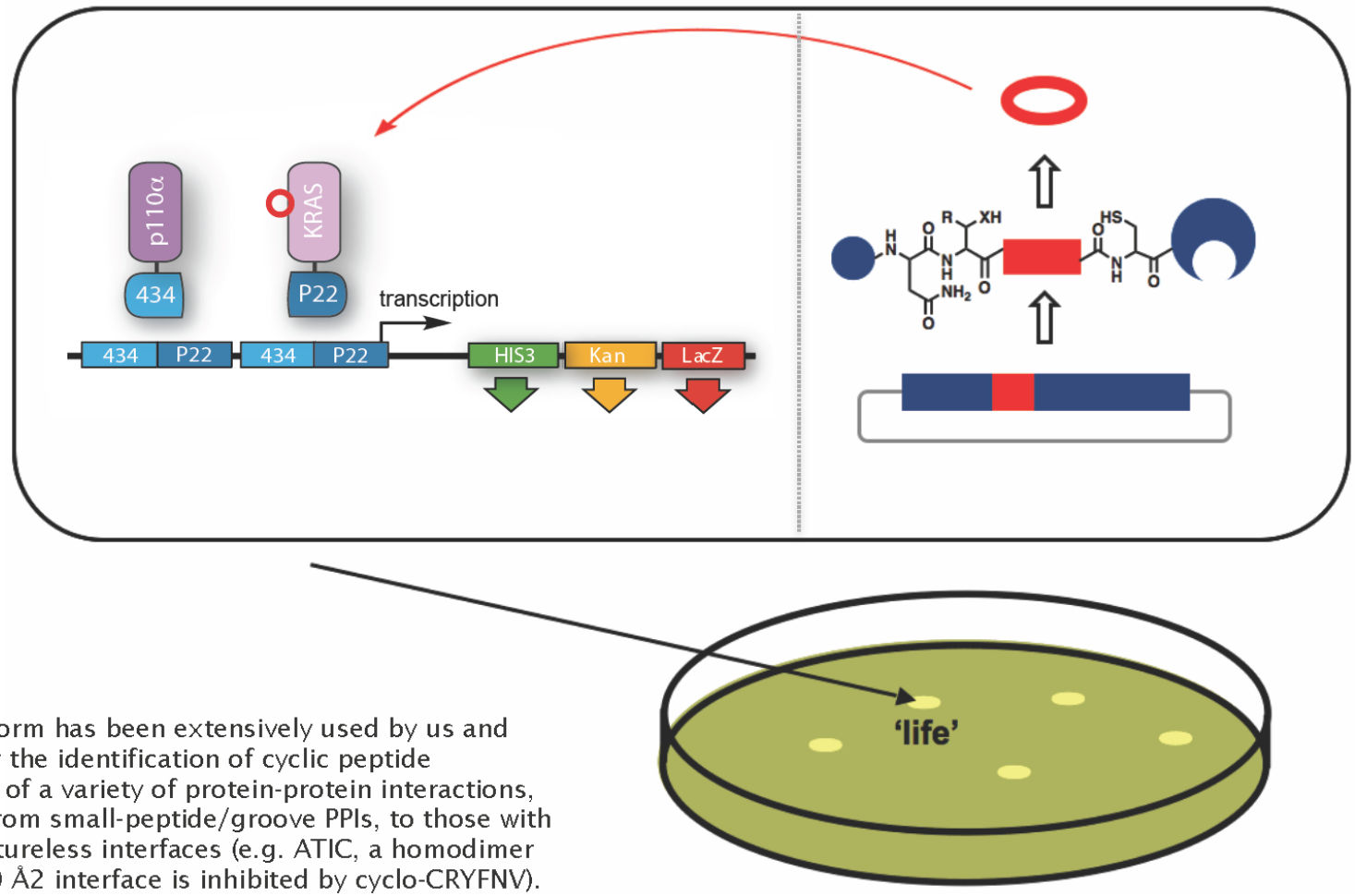
# SICLOPPS library



# Genetic selection of inhibitors



# Genetic selection of inhibitors




- This platform has been extensively used by us and others for the identification of cyclic peptide inhibitors of a variety of protein-protein interactions, ranging from small-peptide/groove PPIs, to those with large, featureless interfaces (e.g. ATIC, a homodimer with 5000 Å2 interface is inhibited by cyclo-CRYFNV).

- Reviewed in K.R. Lennard and A. Tavassoli, 2014, Chem. Eur. J., 20: 10608-10614



**This screening methodology has yielded an ~15nM inhibitor of heterodimerisation of HIF1α/β**

**Ali Tavassoli (2013) JACS 135, 10418**

# Not all effects of disrupting RAS-p110α interaction are tumour cell autonomous

P110α-Mut
P110α-LoxP

CRE-ER (+/-)

P110α-WT
P110α-LoxP

CRE-ER (+/-)

Tamoxifen

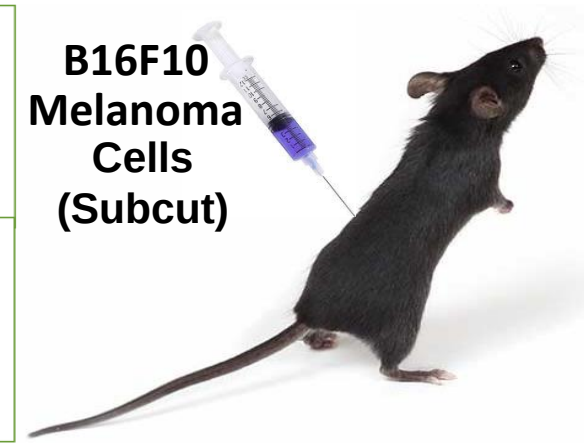
P110α-Mut

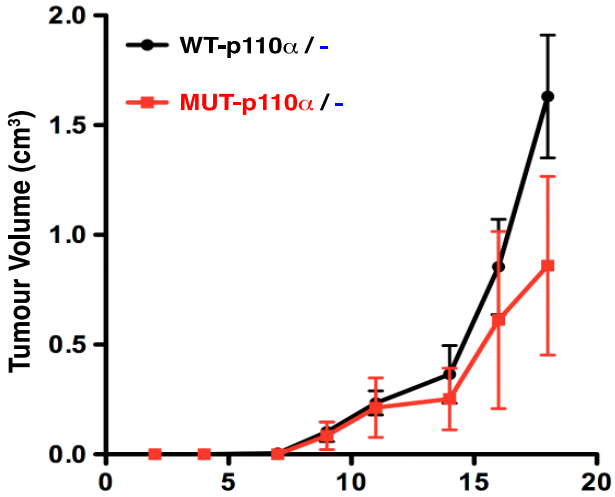
CRE-ER (+/-)

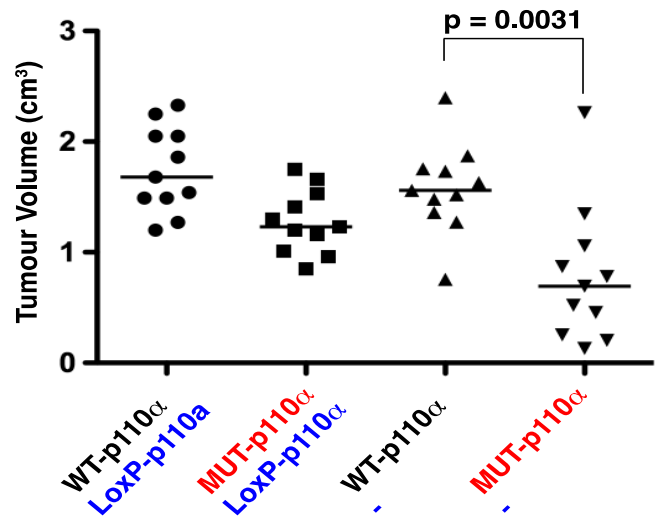
P110α-WT

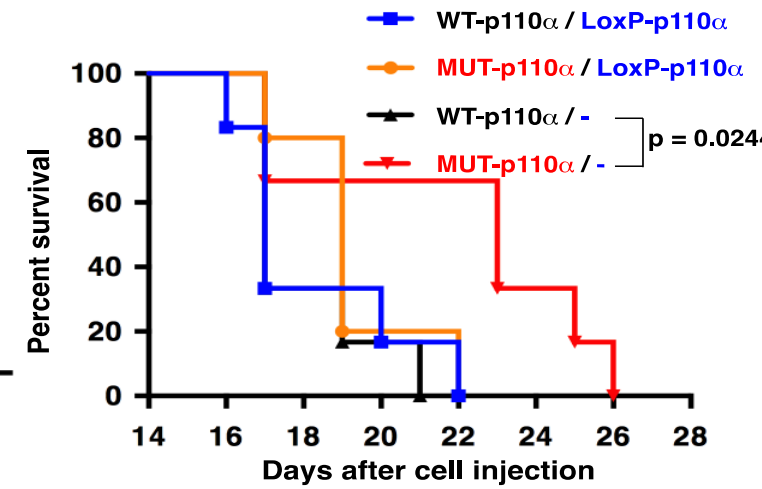
CRE-ER (+/-)

B16F10 Melanoma Cells (Subcut)





# Host RAS-p110α interaction important in supporting metastasis

P110α-Mut
P110α-LoxP

CRE-ER (+/-)

P110α-WT
P110α-LoxP

CRE-ER (+/-)

Tamoxifen

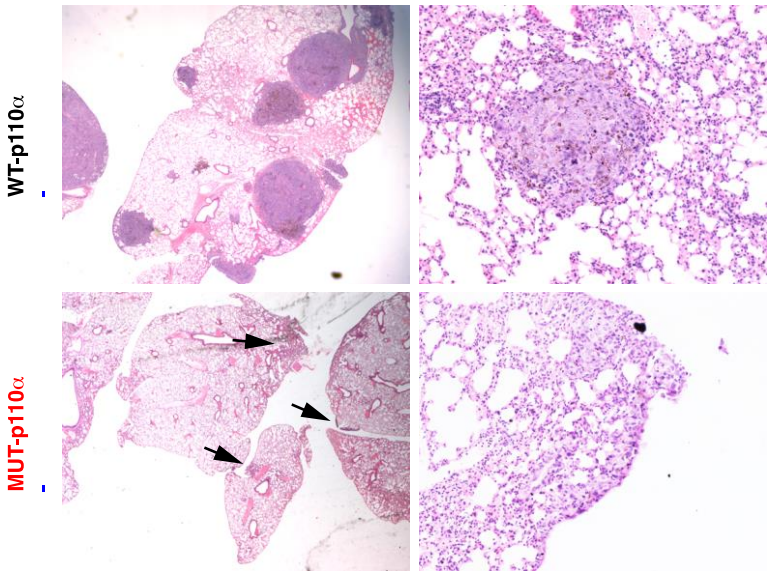
P110α-Mut

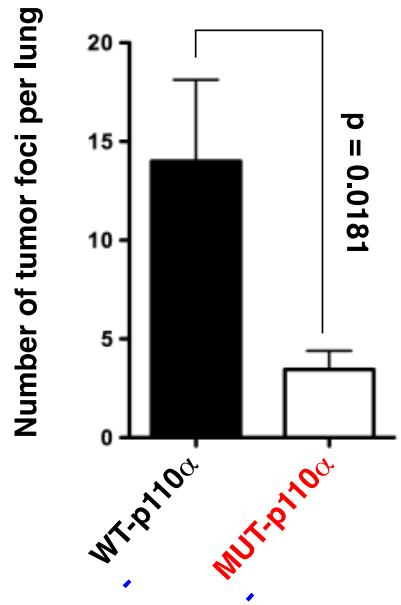
CRE-ER (+/-)

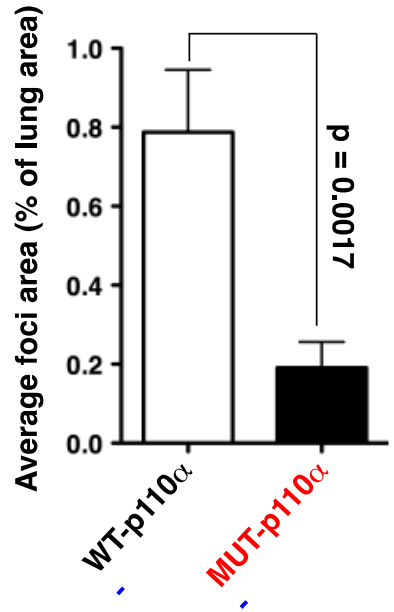
P110α-WT

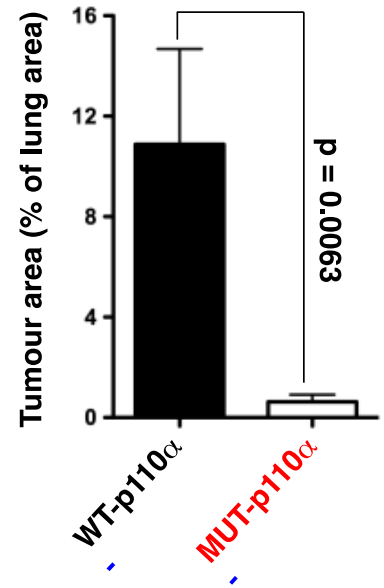
CRE-ER (+/-)

**B16F10 Melanoma Cells (Tail vein)**

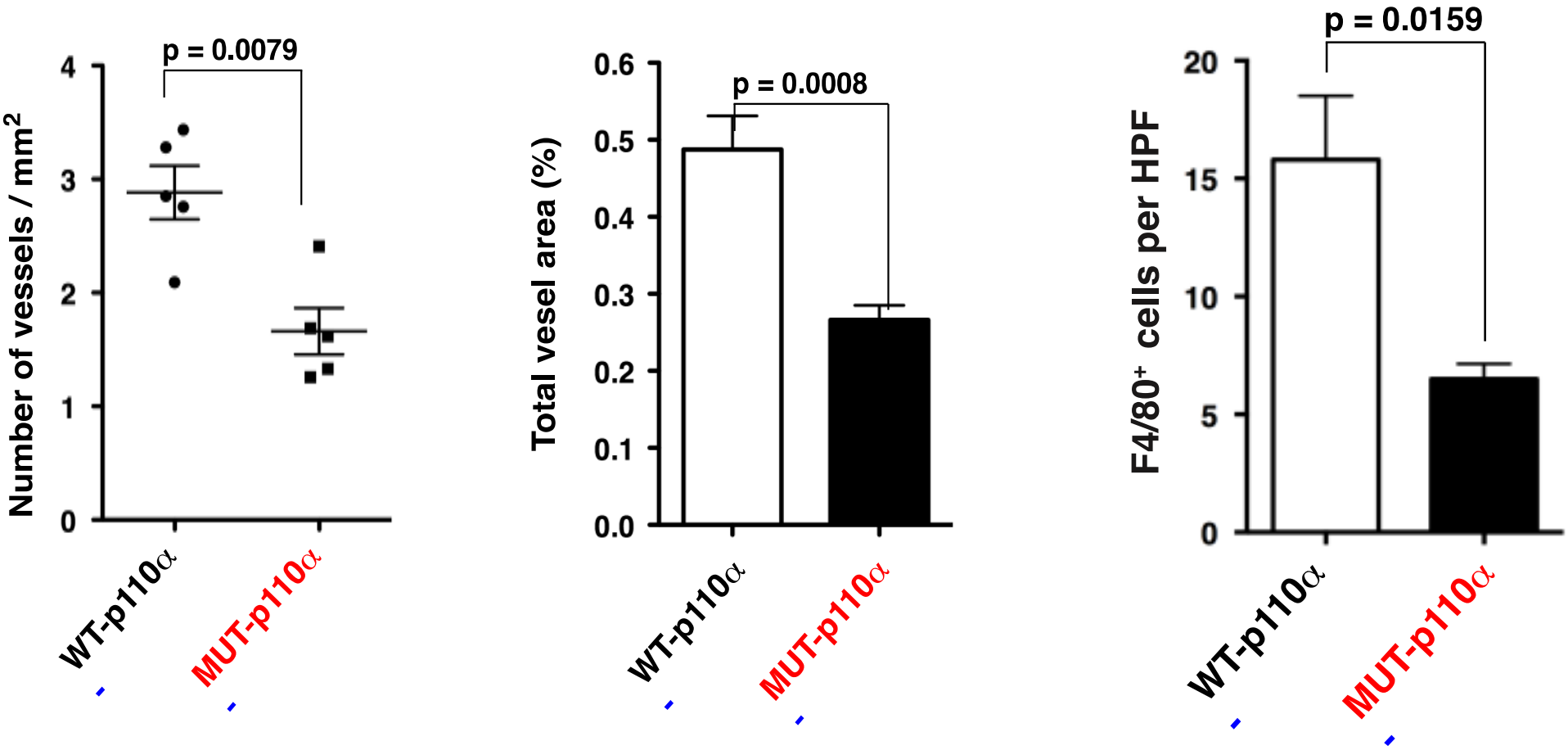


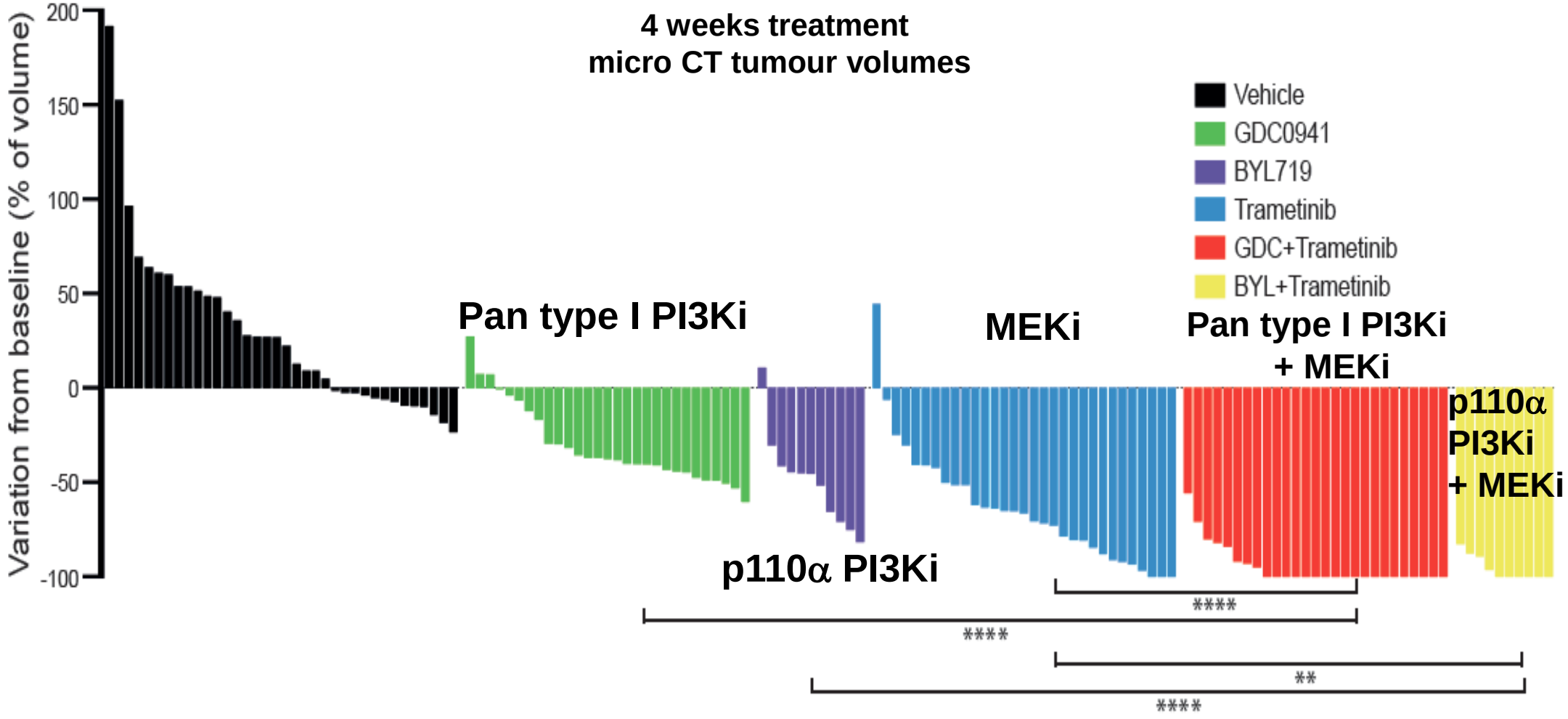



# Loss of host RAS-p110α interaction results in reduced blood vessel density and reduced macrophage numbers in tumours




*RAS interaction with PI3K p110α is required for tumor-induced angiogenesis*
*M.M.Murillo, S.Zelenay, E.Nye, E.Castellano, F.Lassailly, G.Stamp, J.Downward (2014) Journal of Clinical Investigation 124, 3601.*

# Effect of MEK and PI3K inhibitor combination in KRAS LA2 mouse lung tumours



**Combination treatment with MEK and PI3K inhibitors causes marked regression of lung tumours in KRAS LA2 mouse...**

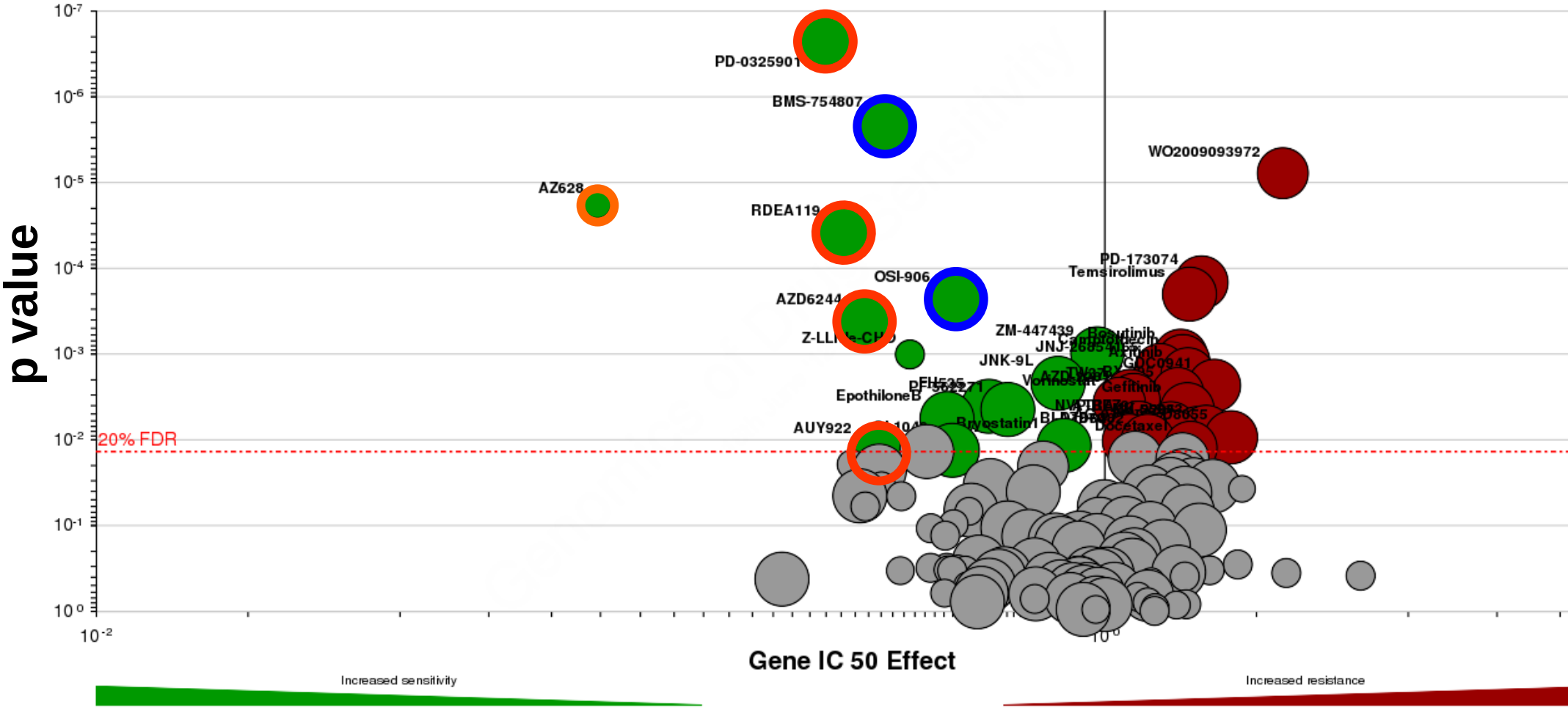
**... however, this is at the cost of very significant toxicity.**

**Is it possible to find  more selective / less toxic approaches to targeting PI3K pathway in RAS mutant cancers?**

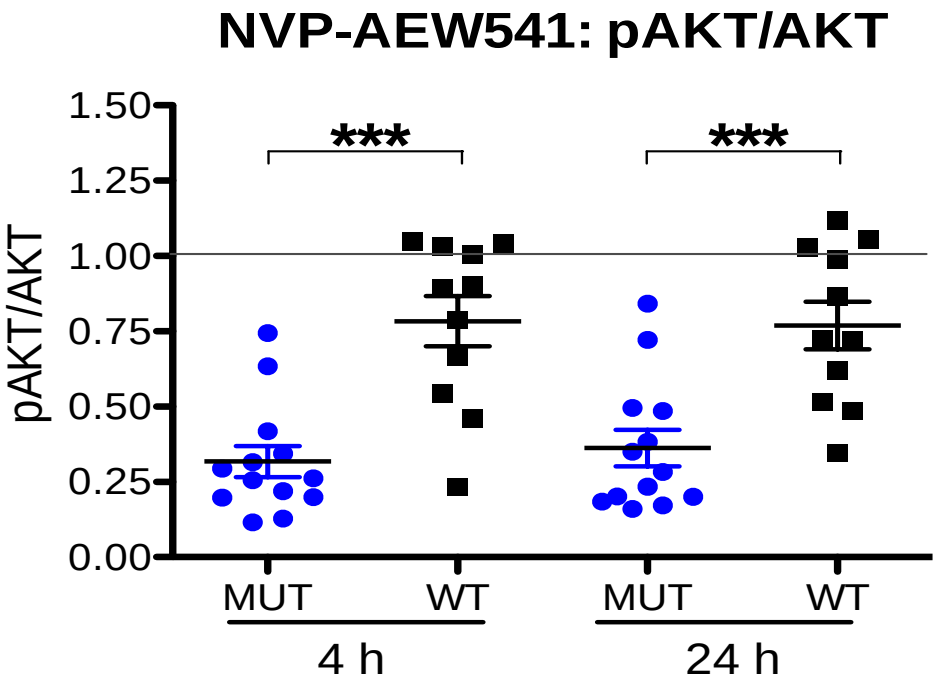
# Drugs selective for KRAS mutant genotype in Sanger/MGH screen

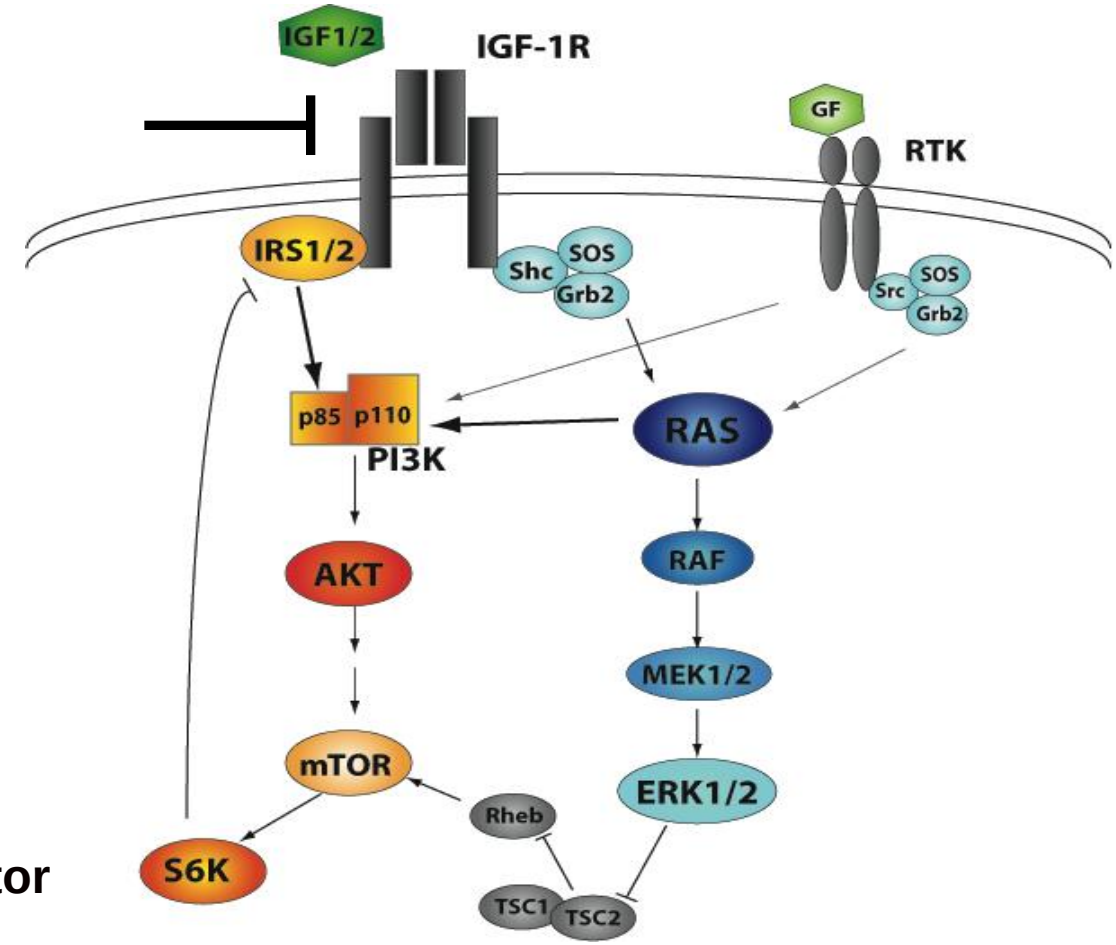
## (The Genomics of Drug Sensitivity in Cancer Project)

## www.cancerrxgene.org



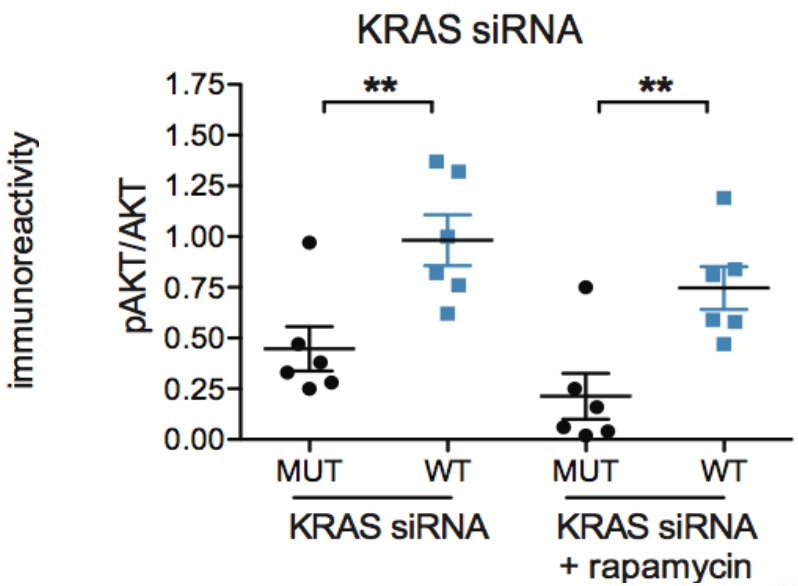
# IGF1R inhibitors inhibit PI3K pathway selectively in RAS mutant cells

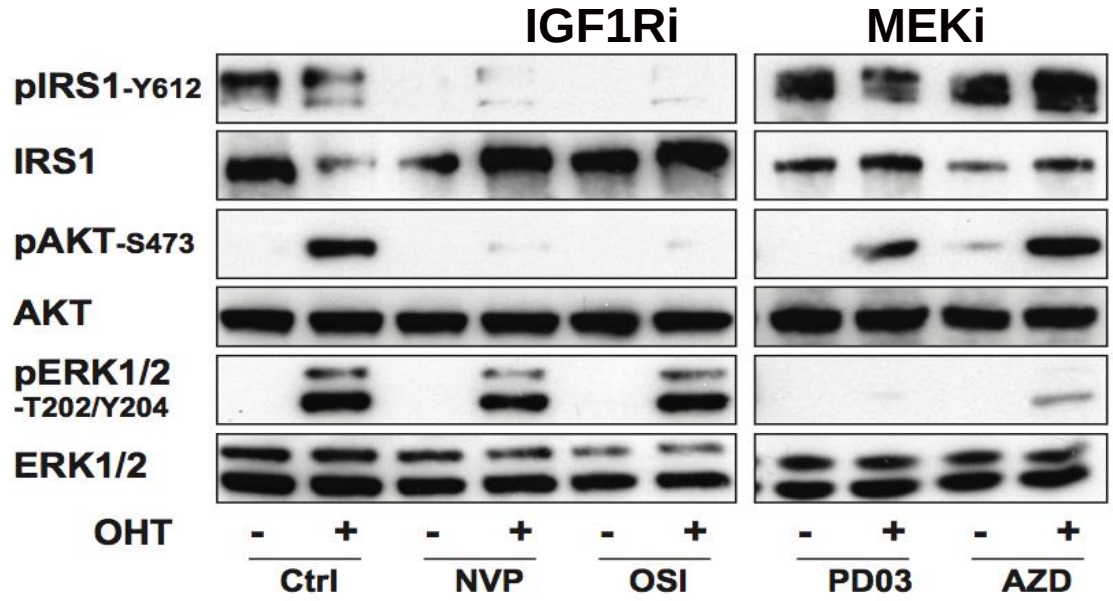


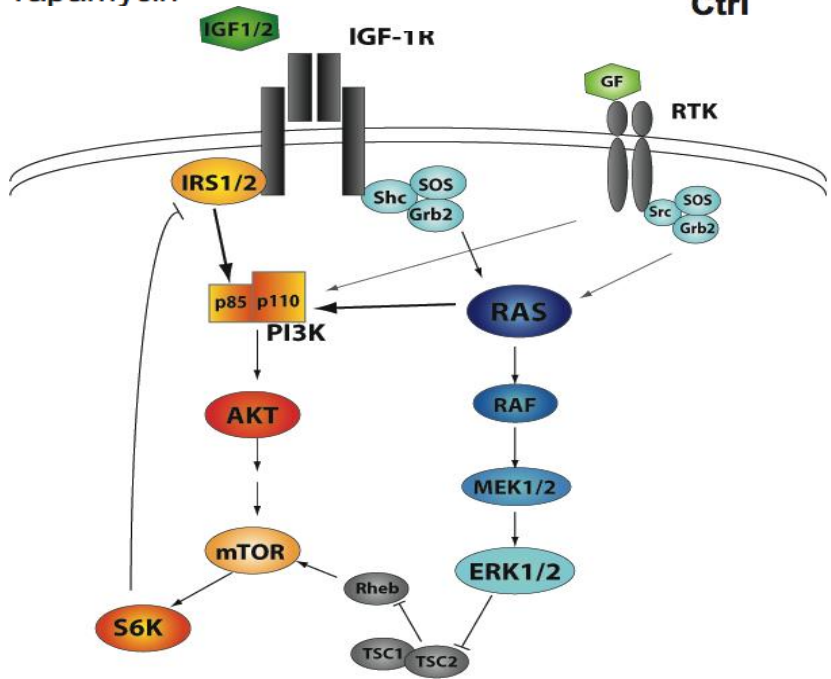

**NVP-AEW541: Novartis IGF1R kinase inhibitor**

# KRAS knockdown inhibits PI3K activity and acute RAS activation stimulates PI3K dependent on basal IGF1R activity




RAS-ER cells

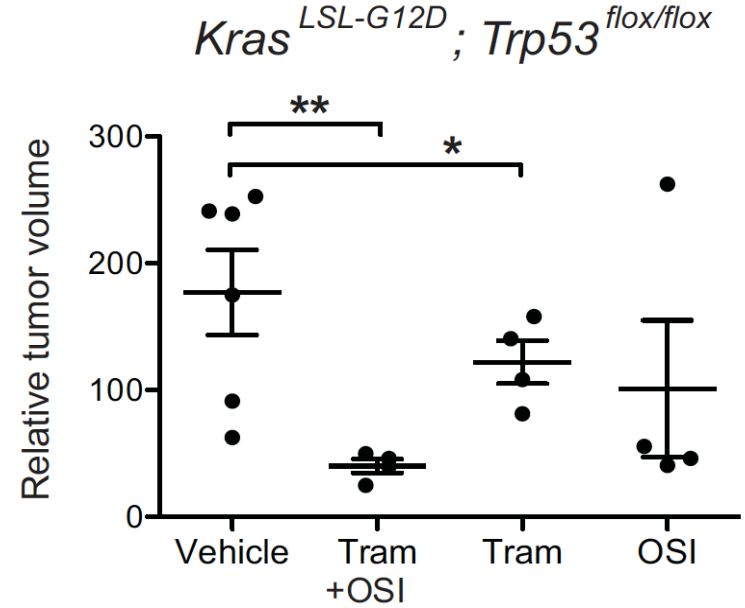


- **In KRAS mutant lung cancer, PI3K stimulation downstream of activated RAS is dependent on basal IGF1R activity**

- **Inhibiting PI3K activity with IGF1R inhibitors may be less damaging to normal cells than the use of PI3K inhibitors**

- **Combined MEK and IGF1R inhibition may be worth exploring in KRAS mutant lung cancer**

- **Early stage trials of combinations of MEK and IGF1R inhibitors ongoing**




**KRAS p53 mutant lung cancer mouse model**
**15 days treatment with trametinib (2.5mg/kg/d) plus linsitinib/OSI-906 (40mg/kg/d)**

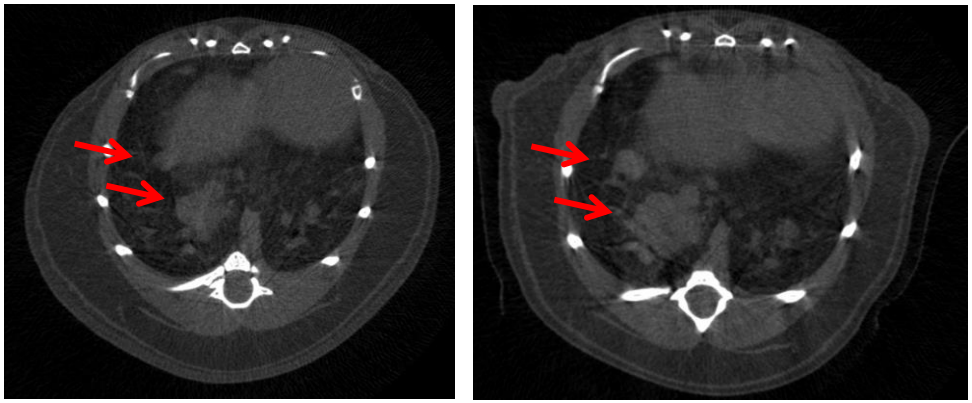
*M. Molina-Arcas, D.C. Hancock, C. Sheridan, M.S. Kumar, J. Downward (2013) Cancer Discovery 3, 548-563. "Coordinate direct input of both KRAS and IGF1 receptor to activation of PI 3-kinase in KRAS mutant lung cancer."*

# How can major but transient regressions be upgraded to cures?

## - Can targeted therapies be combined with immune checkpoint blockade?

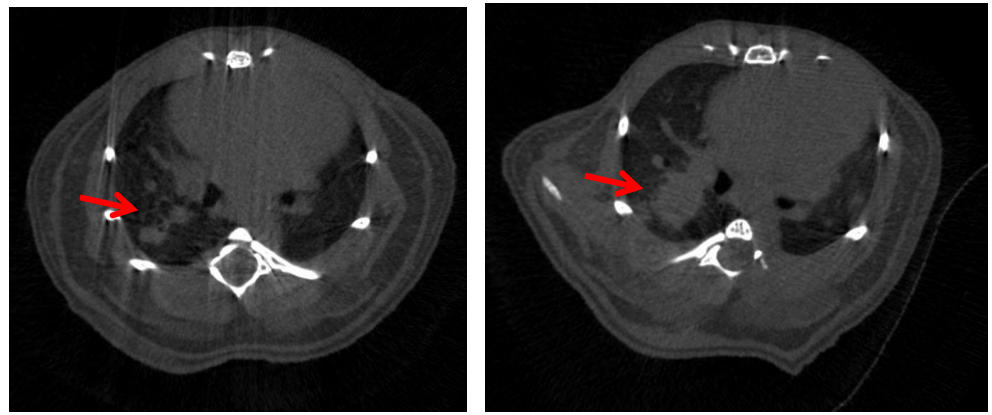
### PD-L1 blockade has no anti-tumour activity in $Kras^{LSLG12D/+};Trp53^{Fl/Fl}$ mice

Isotype



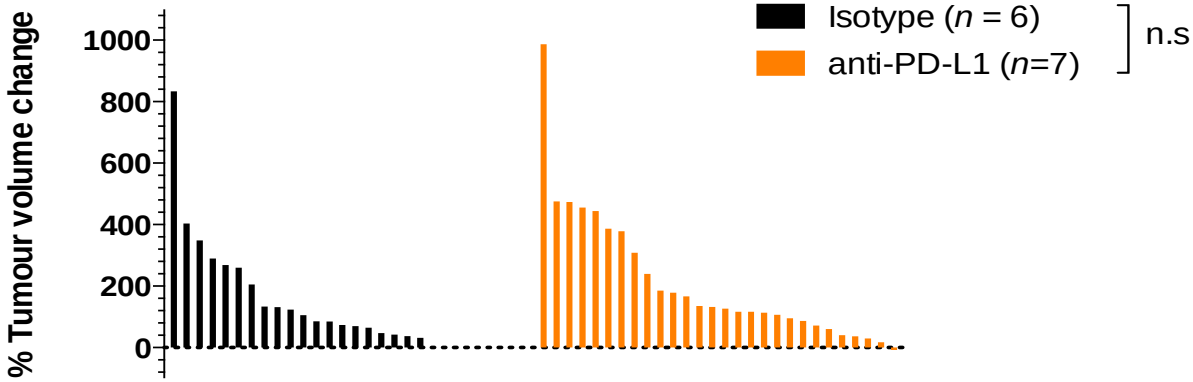
Week 0 Week 2

anti-PD-L1

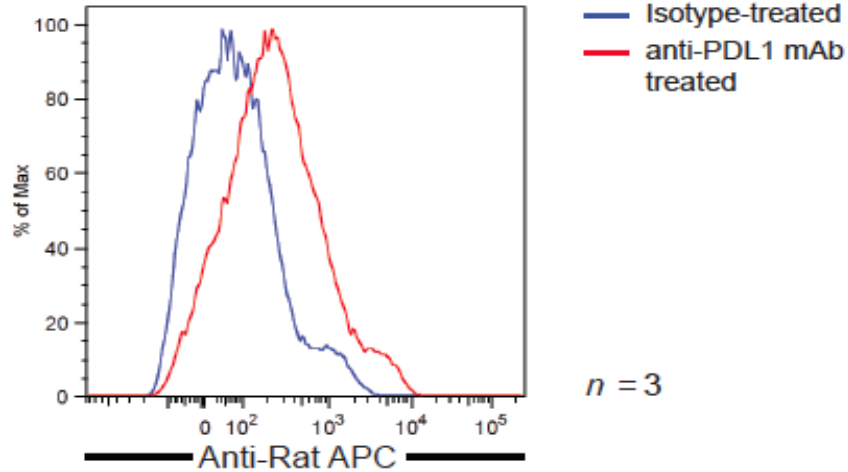


Week 0 Week 2

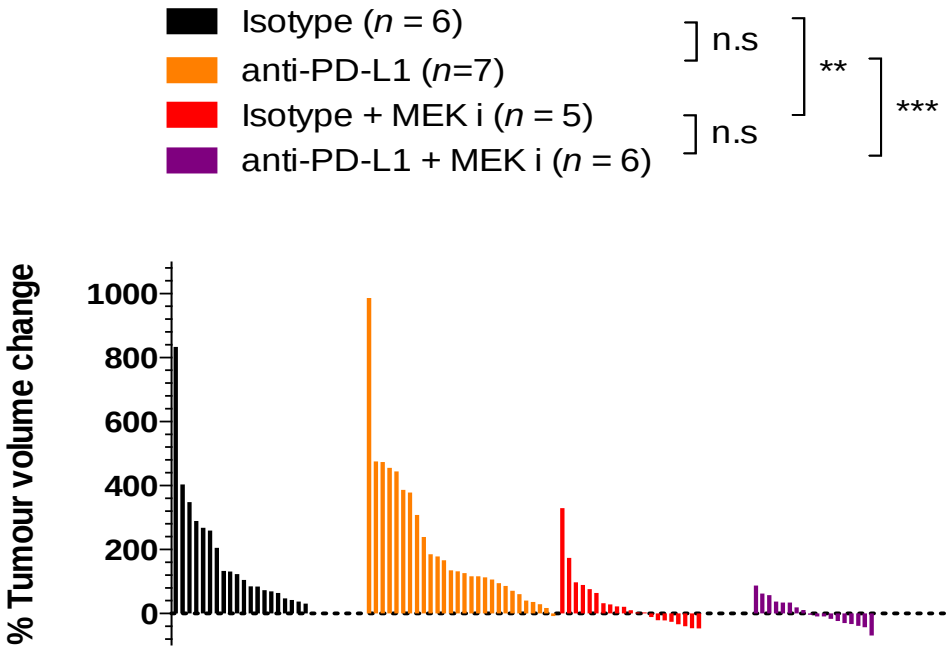
2 weeks on therapy – volume change

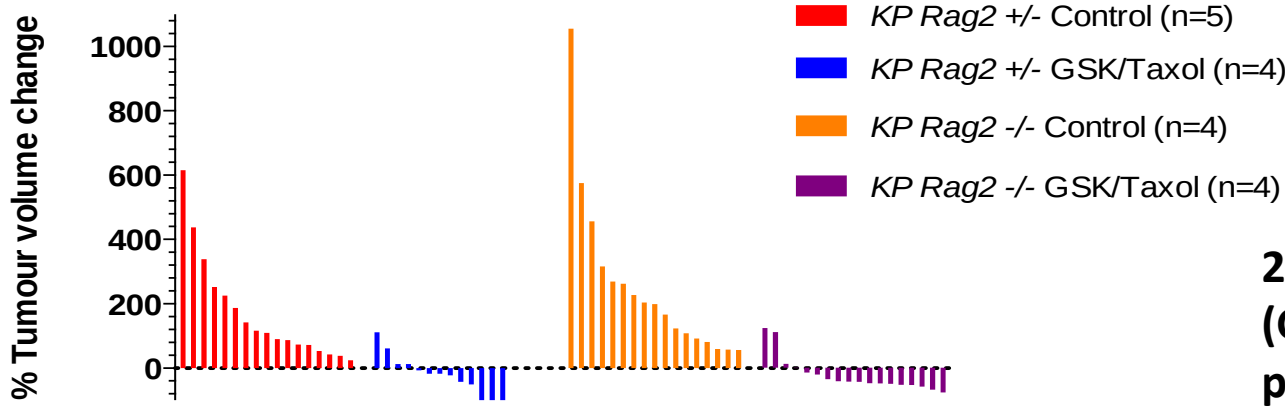



Lung tissue PD-L1 mAb binding



# Lack of adaptive immune response to Kras p53 mouse model tumours




**2 weeks on therapy, MEKi (GSK1120212/trametinib) plus taxol**

**GEMMs such as the KP mouse lung cancer model have tumours with <5 coding mutations compared to ~500 non-silent coding mutations in human lung cancer**

**Need better mouse models to study the interplay of the immune system and the tumour in the response to therapies, whether chemo, targeted agents or immunotherapies**

# Therapy of KRAS mutant cancers

- **MEK inhibitors are the only agents with proven, albeit modest, KRAS genotype selectivity in the clinic.**
- **MEK inhibitors promising as scaffold for combination, e.g. with IGF1R inhibitors or with proteasome inhibitors, also cytotoxics**
- **Synthetic lethal approach points to combined inhibition of proteasome and ROCK (Steckel 2012, Kumar 2012). However, ROCK inhibitors very early stage in clinic.**
- **Immune checkpoint therapies may have potential in KRAS mutant cancers, although genotype selectivity unlikely. Current KRAS GEMMs fail to model adequately**

# Acknowledgements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Davide Zecchin
Alexandra Pender
Maggie Cheung
Daniël Lionarons
Esther Castellano

ICR The Institute of Cancer Research

LRI Pathology
Gordon Stamp
Emma Nye
LRI In Vivo Imaging
Francois Lassailly
May Zaw Thin
LRI High Throughput Screening
Mike Howell
Ming Jian
Becky Saunders
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LRI Advanced Sequencing
Nik Matthews
Adam Rabinowitz

Southampton
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SEVENTH FRAMEWORK PROGRAMME

AICR
CANCER KNOWS NO BOUNDARIES
FORTUNATELY, NEITHER DO WE