**Heterogeneity among early-stage K-Ras driven lung adenocarcinoma predicts tumour aggressiveness and identifies novel therapeutic targets**

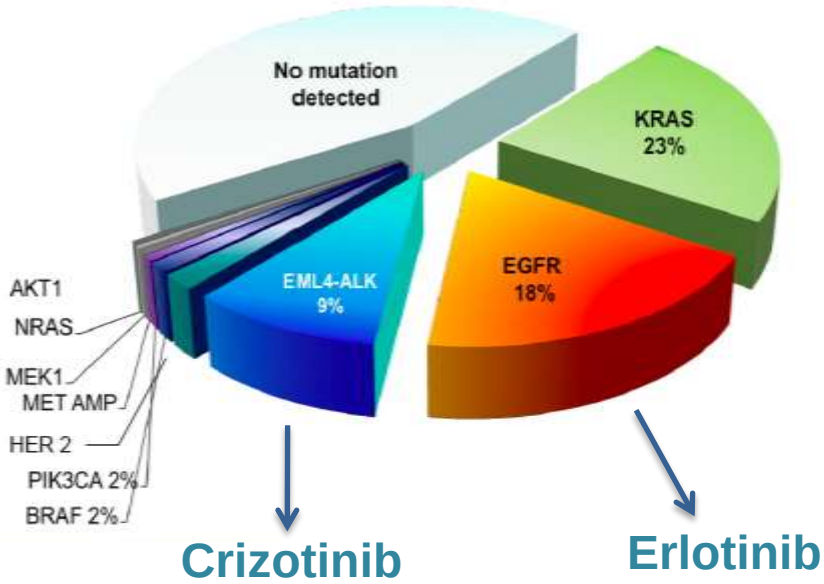
cnio Centro Nacional de Investigaciones Oncológicas

Canceropole 17.10.14

## **Ras Mutations in Human Tumors** (COSMIC, 2013)

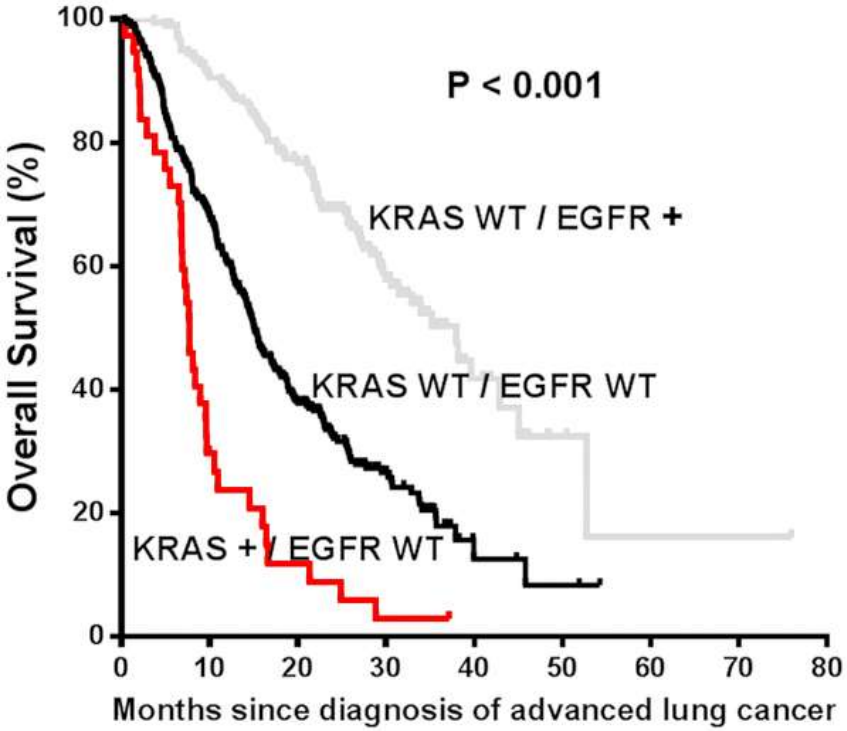
| Tissue/Organ | K-Ras | N-Ras | H-Ras |
|---|---|---|---|
| **TOTAL: Samples tested** | **130,500** | **56,300** | **33,500** |
| **TOTAL: Percentage** | **22%** | **6%** | **3%** |
| **Pancreas** | **58%** | 1% | 0% |
| **Intestine/Colon** | **28%** | 2% | <1% |
| **Billiary tract** | **26%** | 2% | 1% |
| **Lung** | **23%** | 1% | 1% |
| **Ovary** | **12%** | 1% | 0% |
| Endometrium | 15% | 3% | <1% |
| All Others | 0-7% | 0-15% | 0-12% |

# *Introduction: lung cancer mutations*



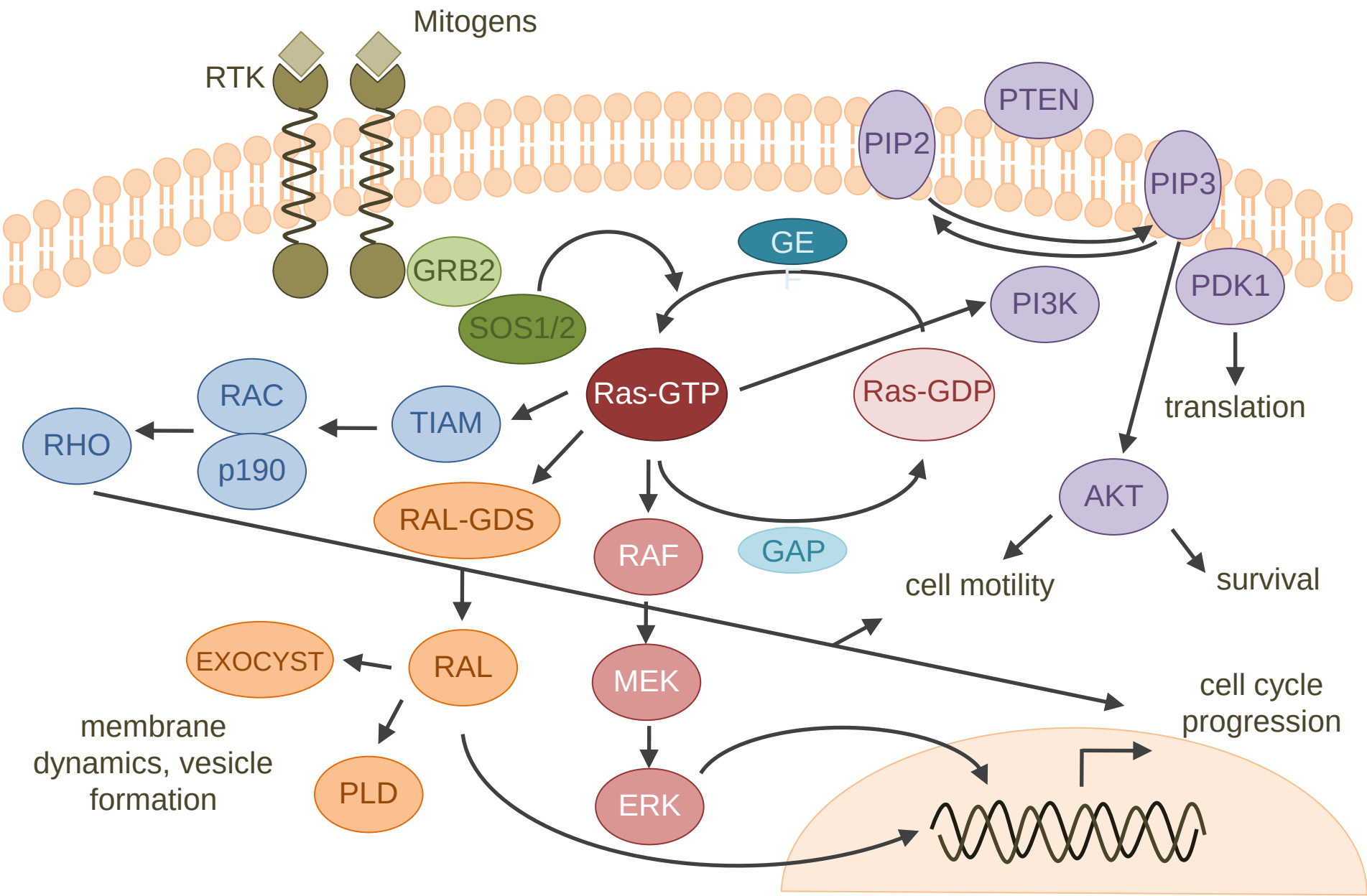

→ **NO TARGETED THERAPY**

Rudin, C, *et al*. (2012)

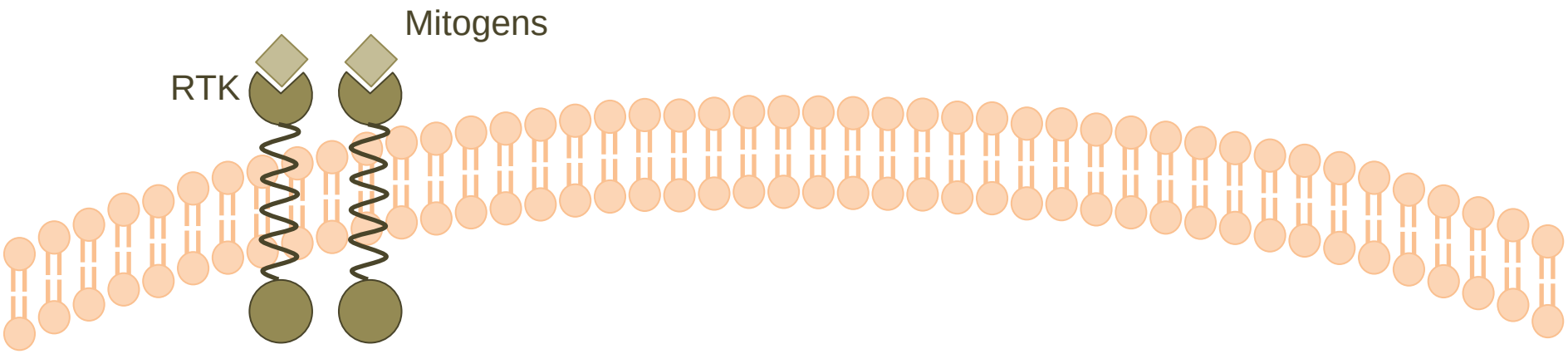


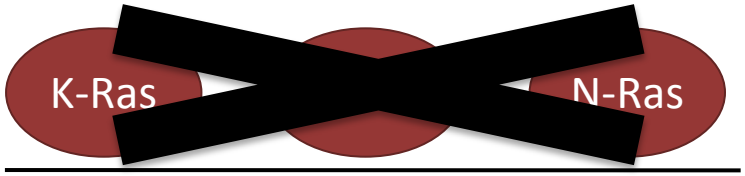

Sun, JM, *et al*. (2013)

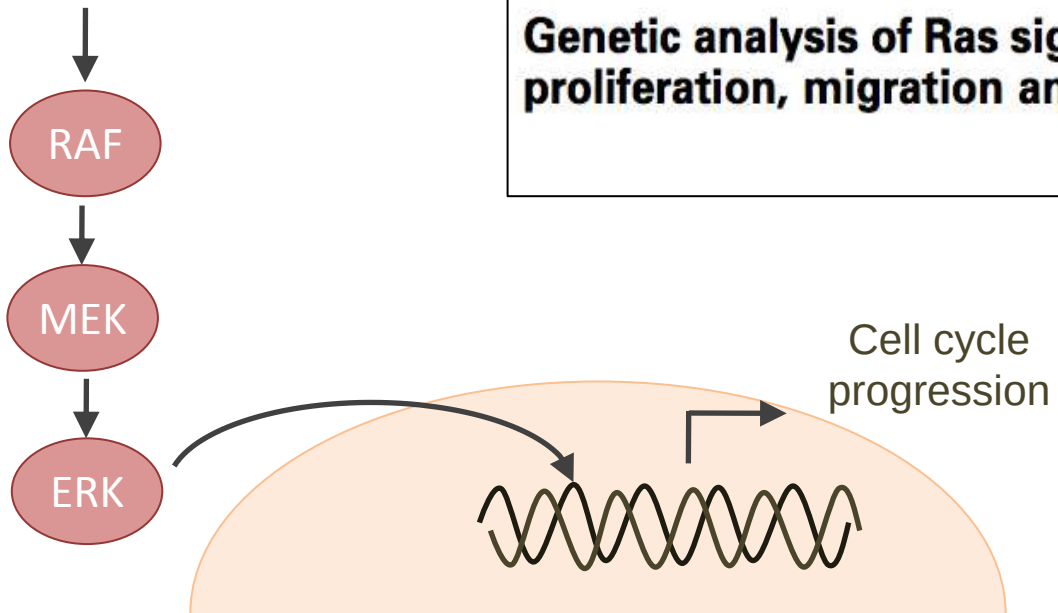
# *Introduction: Ras activation*



# *Targeting MAPK signalling*




The EMBO Journal (2010) 29, 1091–1104 | © 2010 European Molecular Biology Organization | All Rights Reserve
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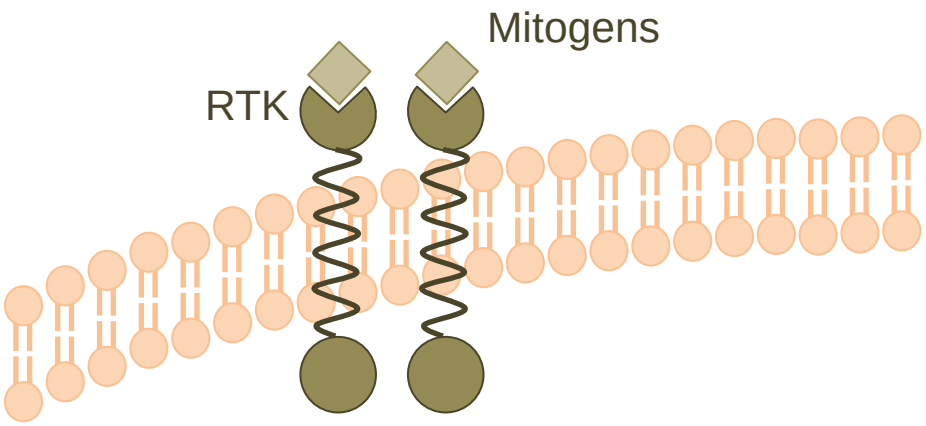
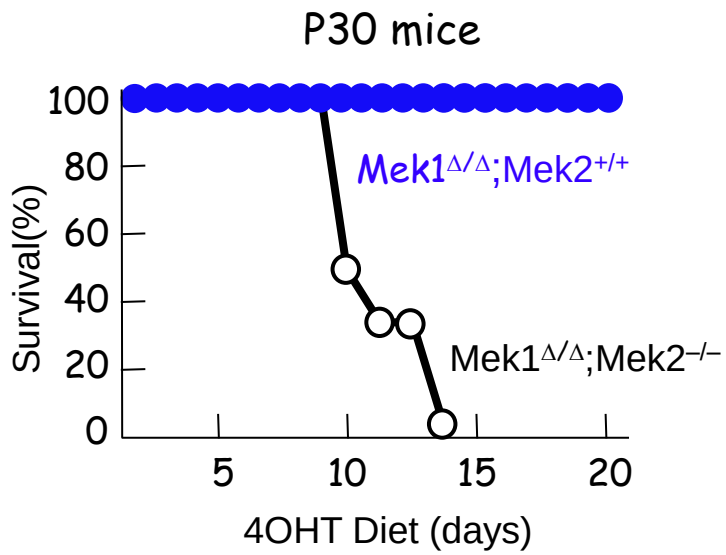
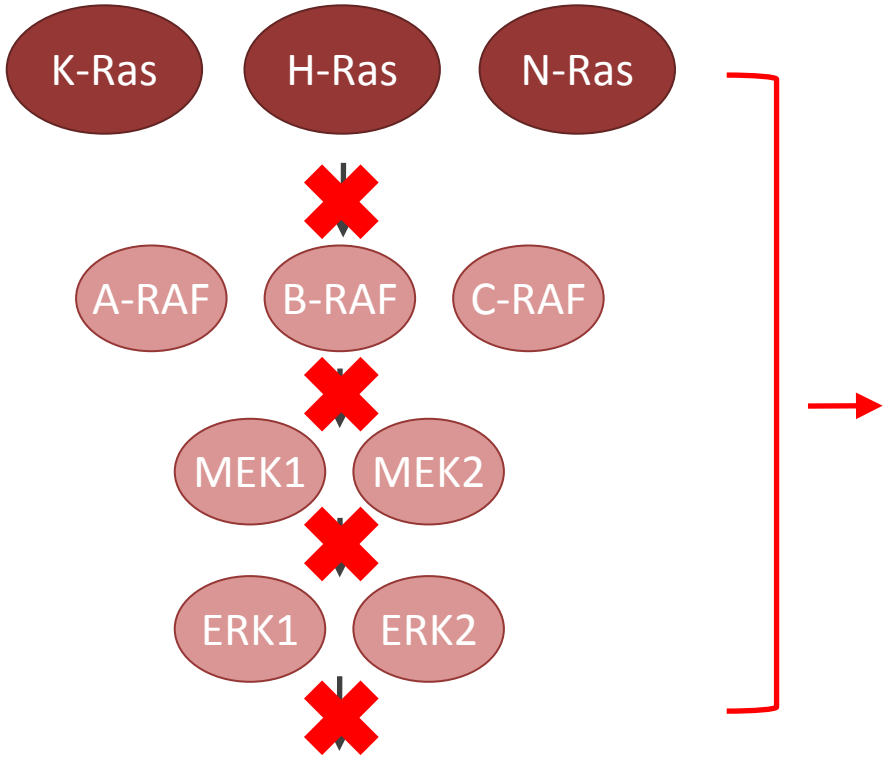
THE EMBO JOURNAL

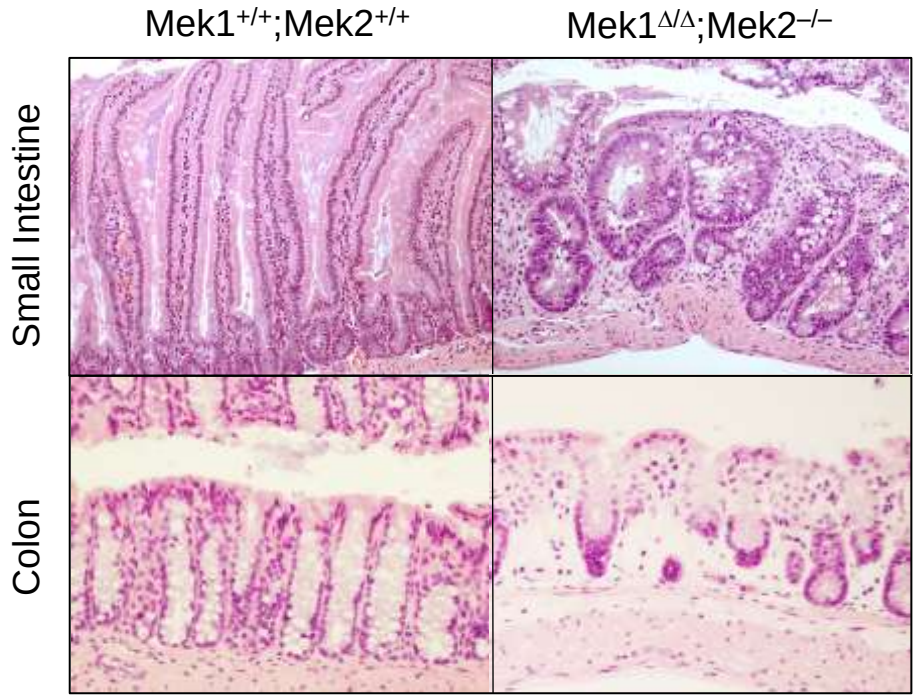
**Genetic analysis of Ras signalling pathways in cell proliferation, migration and survival**

# *Targeting MAPK signalling*

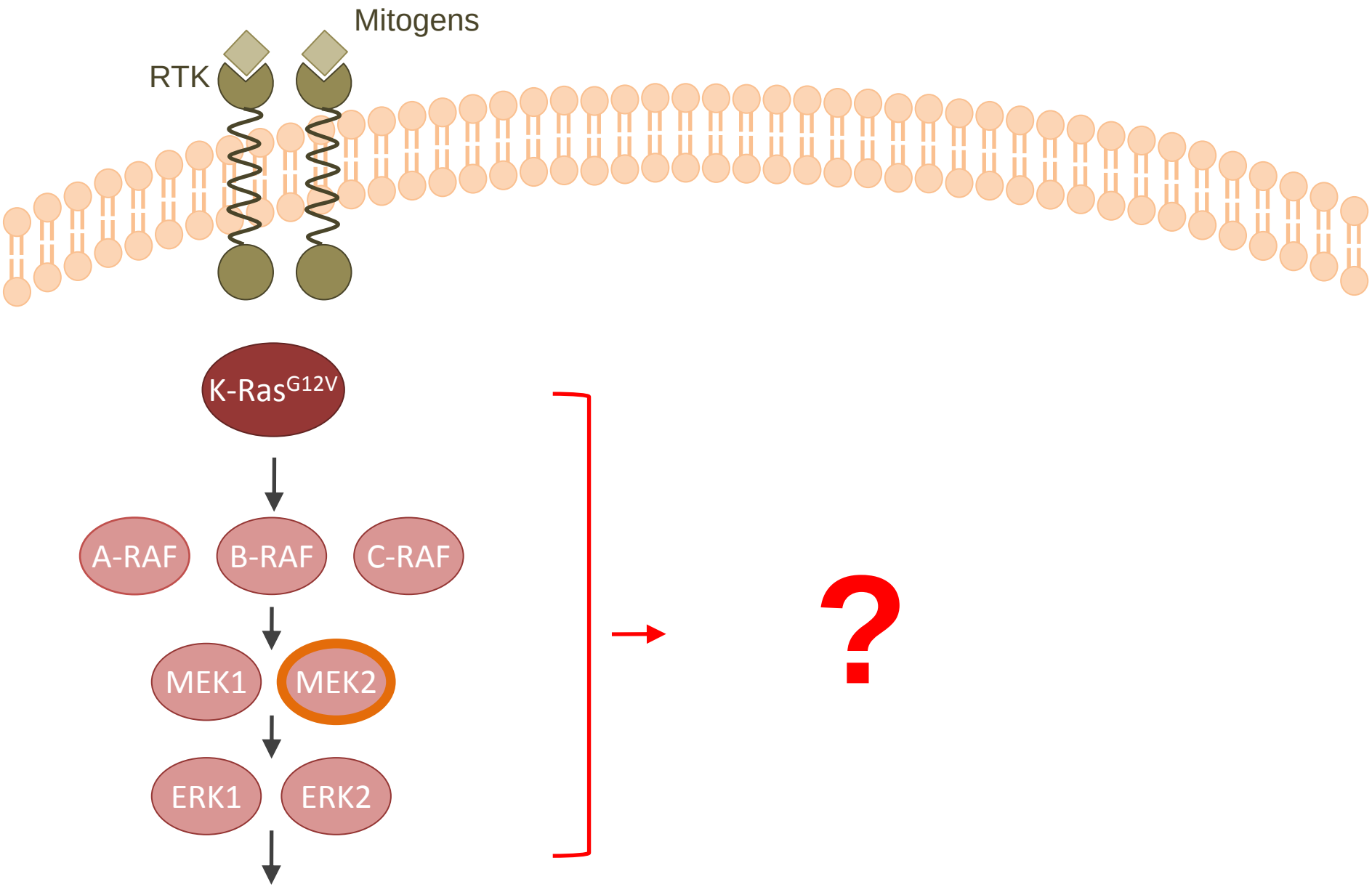


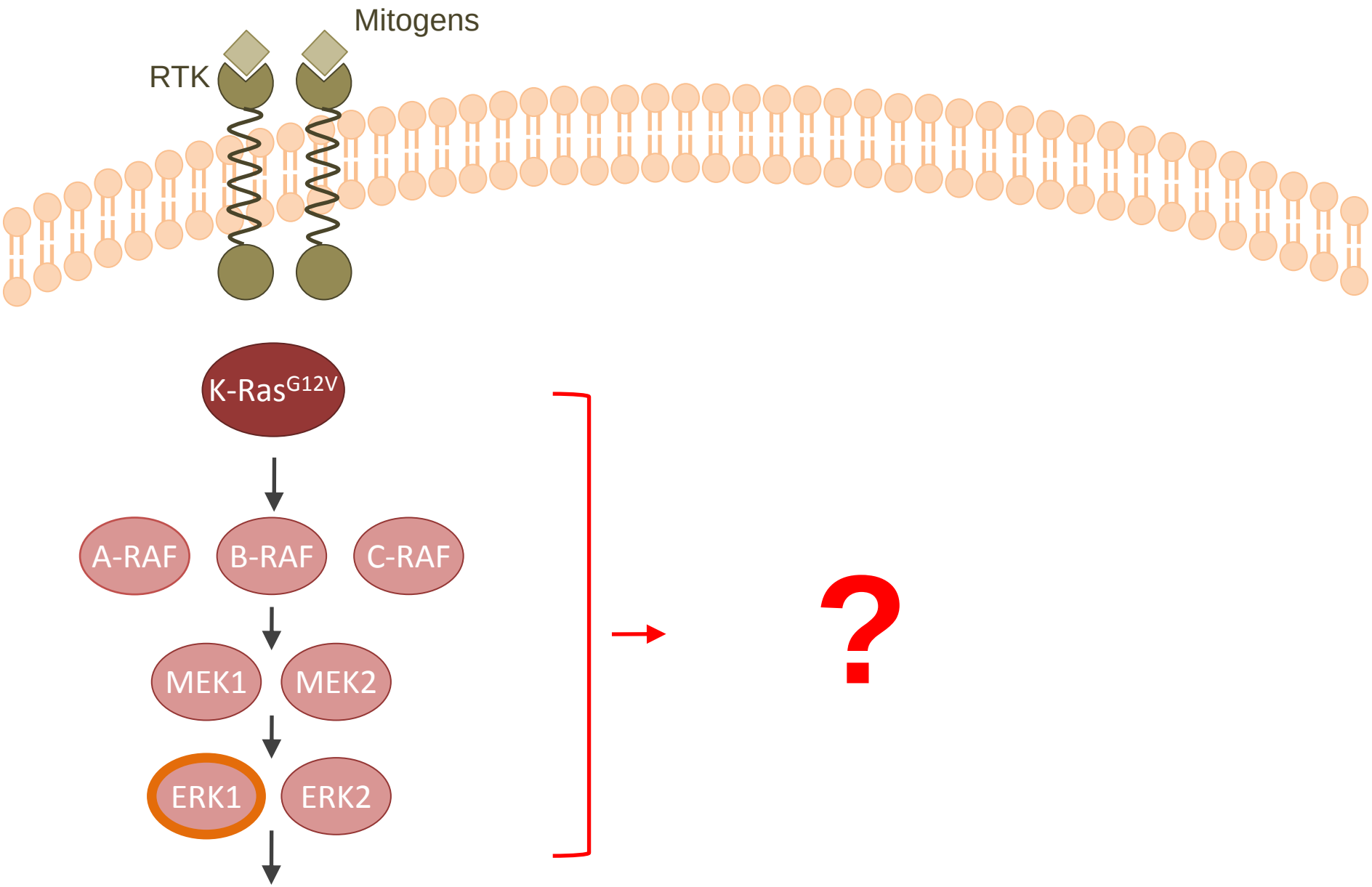



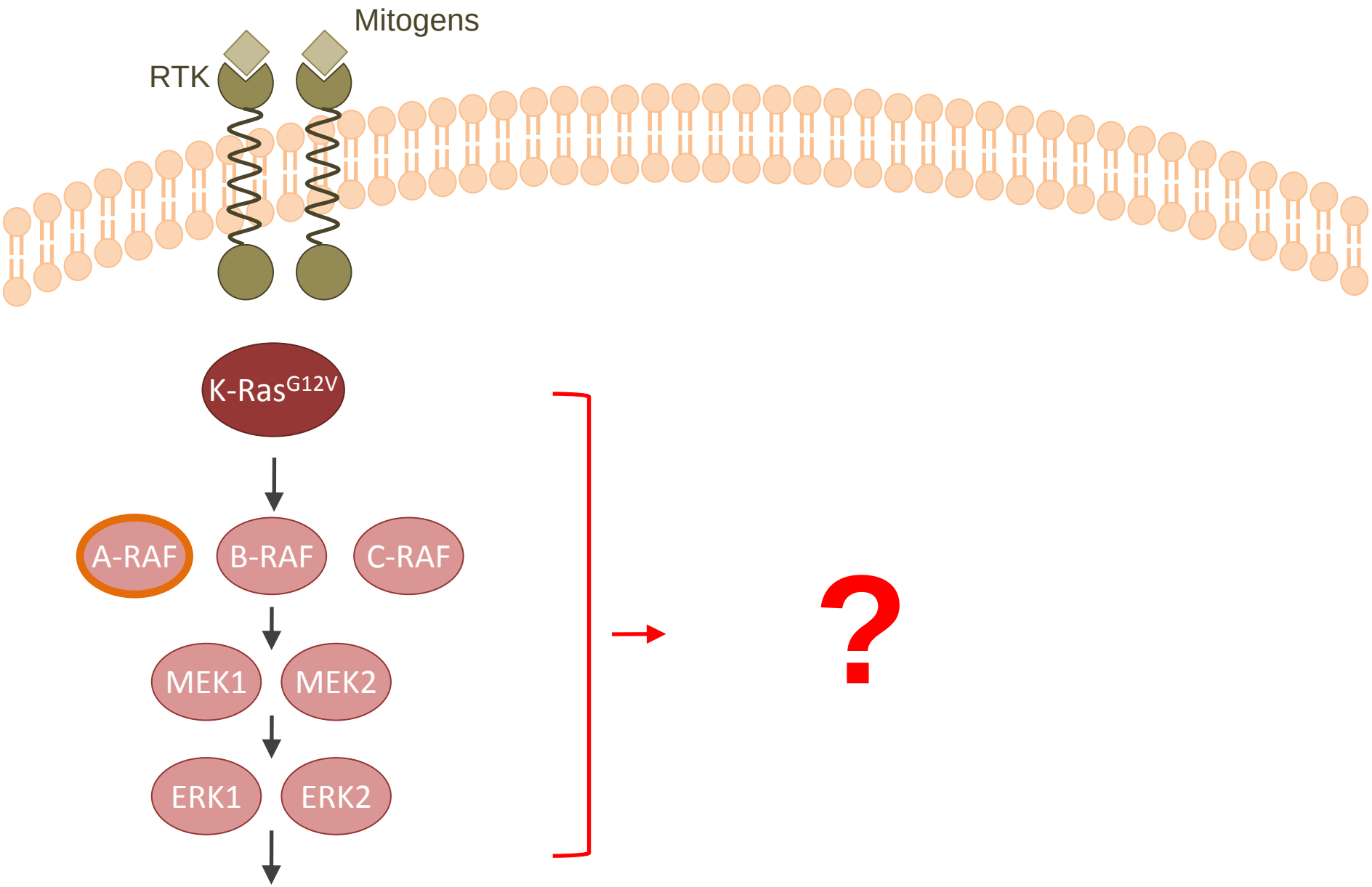
# *Targeting MAPK signalling*



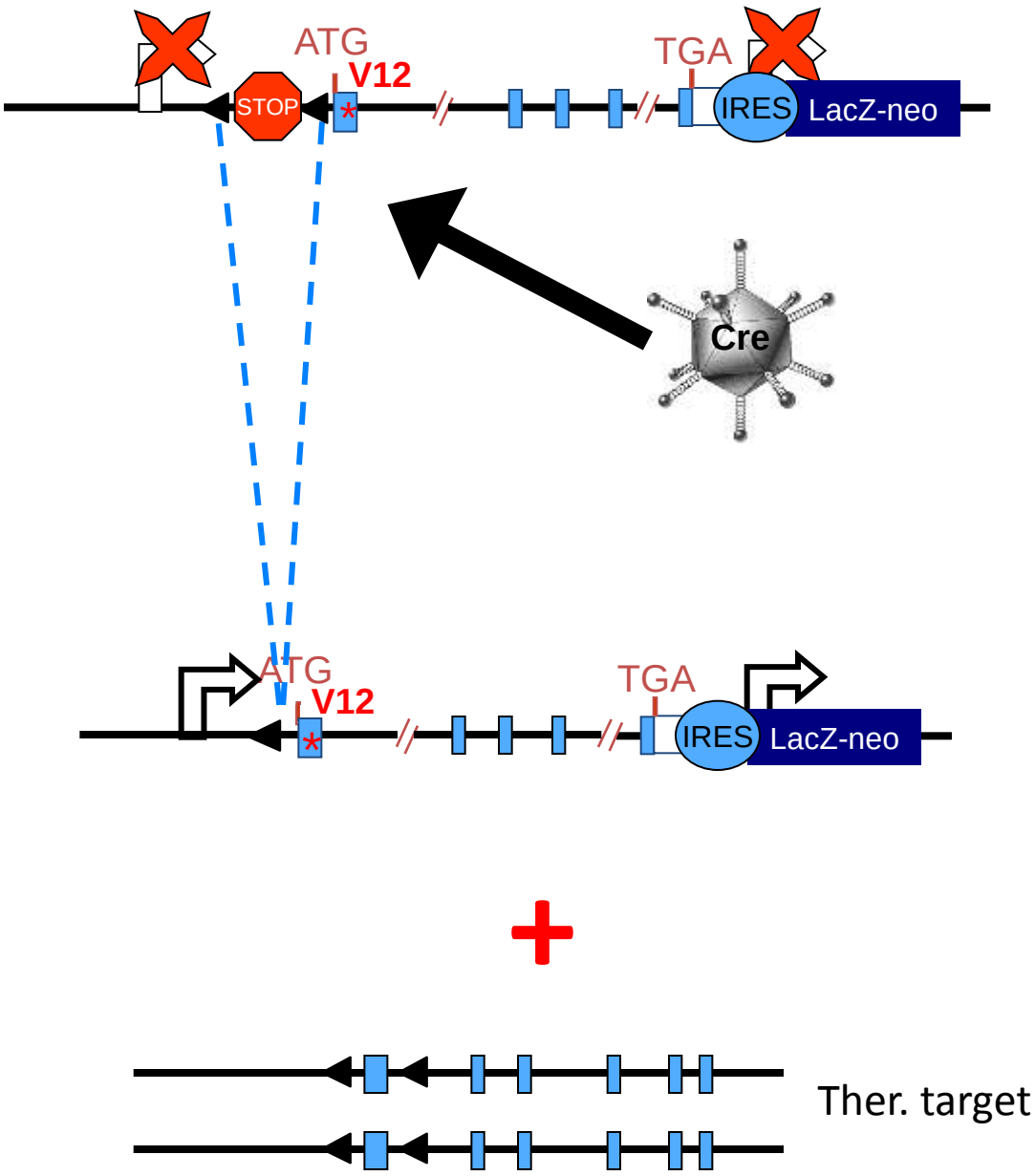
# *Targeting MAPK signalling*

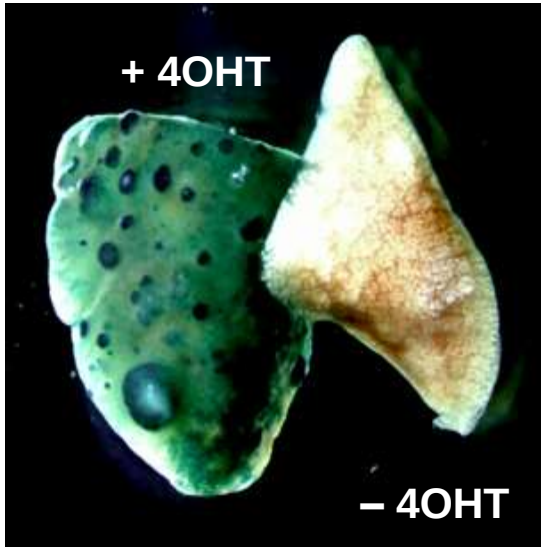


# *Targeting MAPK signalling*

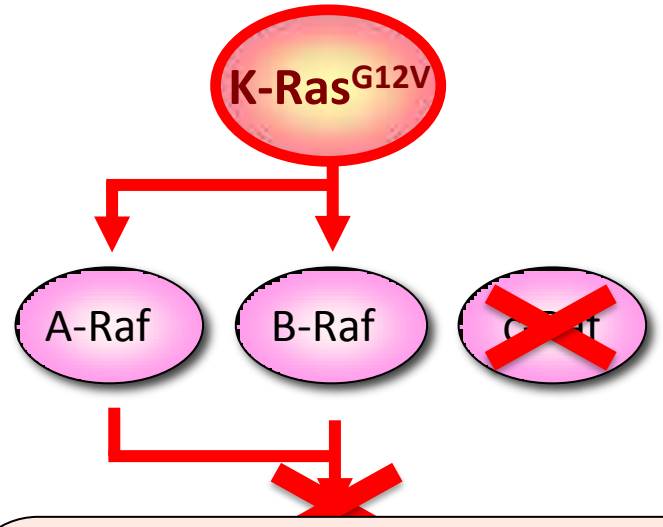


# *Targeting the cell cycle*

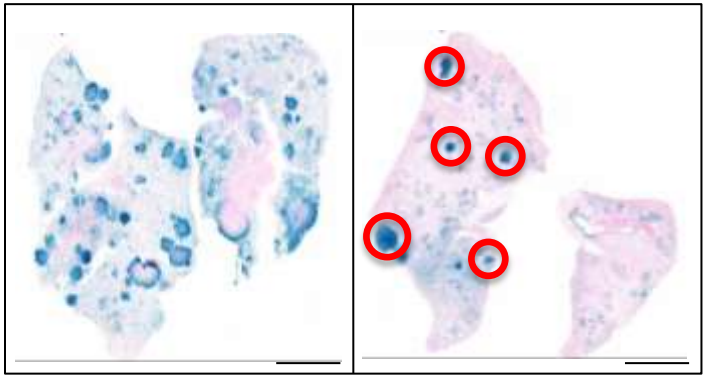


# *Target validation: Raf kinases*

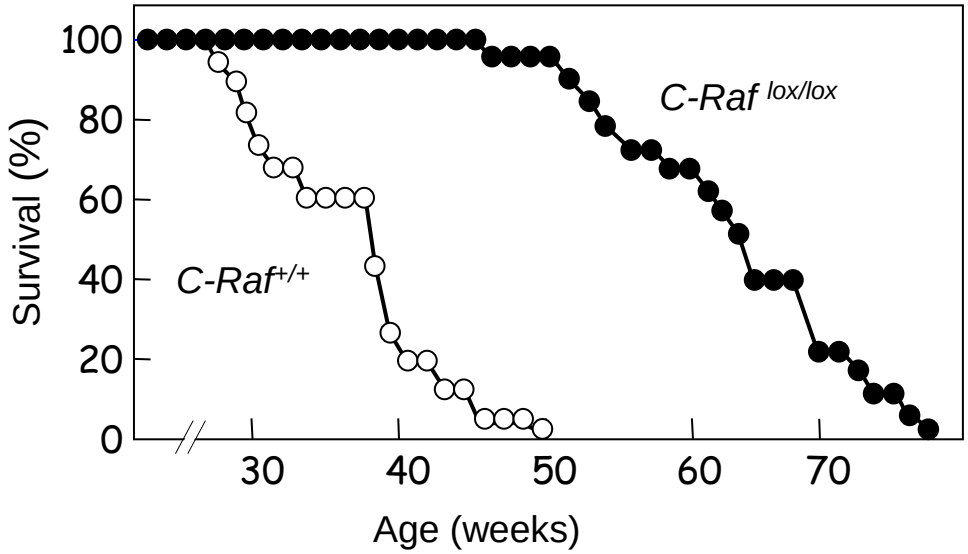


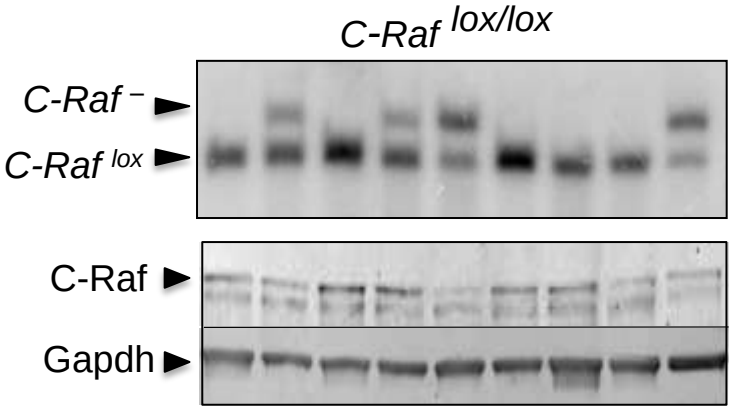

Tumor Burden at 6 months of age



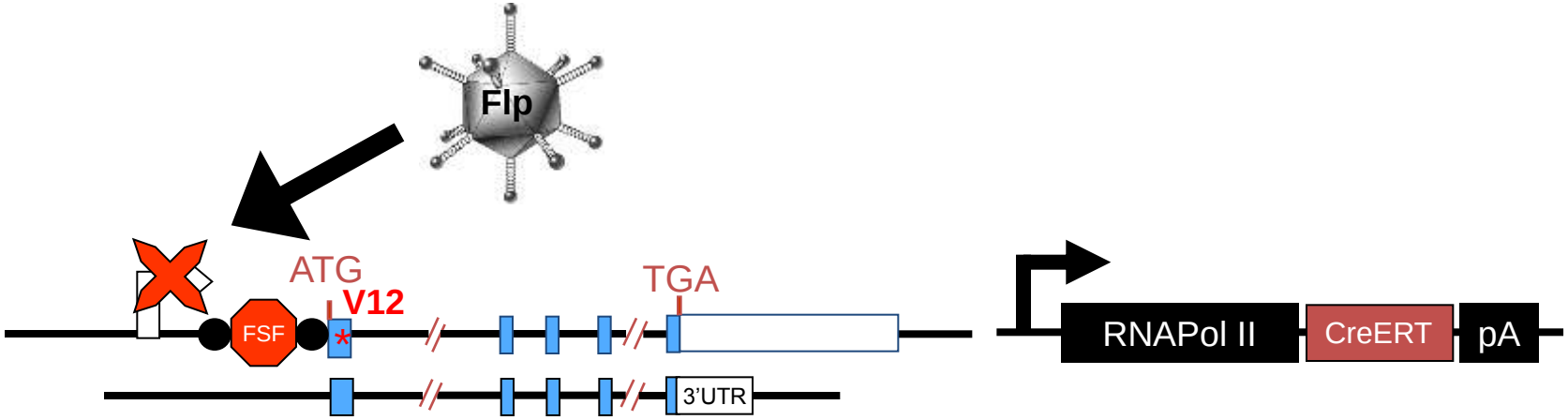
*C-Raf* $^{+/+}$ *C-Raf* $^{lox/lox}$

C-Raf, but not B-Raf or A-Raf, is essential for initiation of K-Ras$^{G12V}$ oncogene-driven lung adenocarcinomas, yet dispensable for adult homeostasis



# *MAPK: Therapeutic approach*



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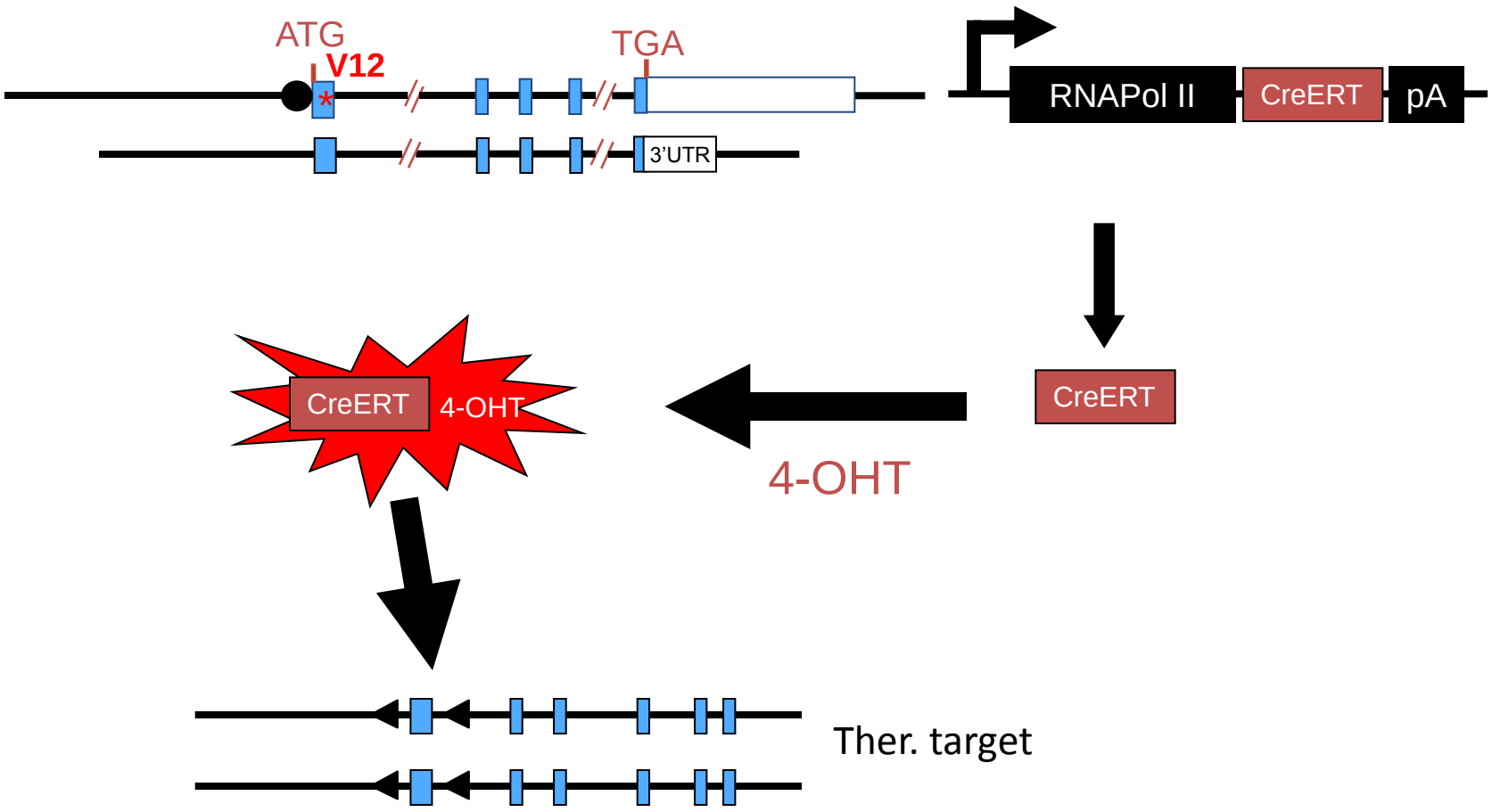
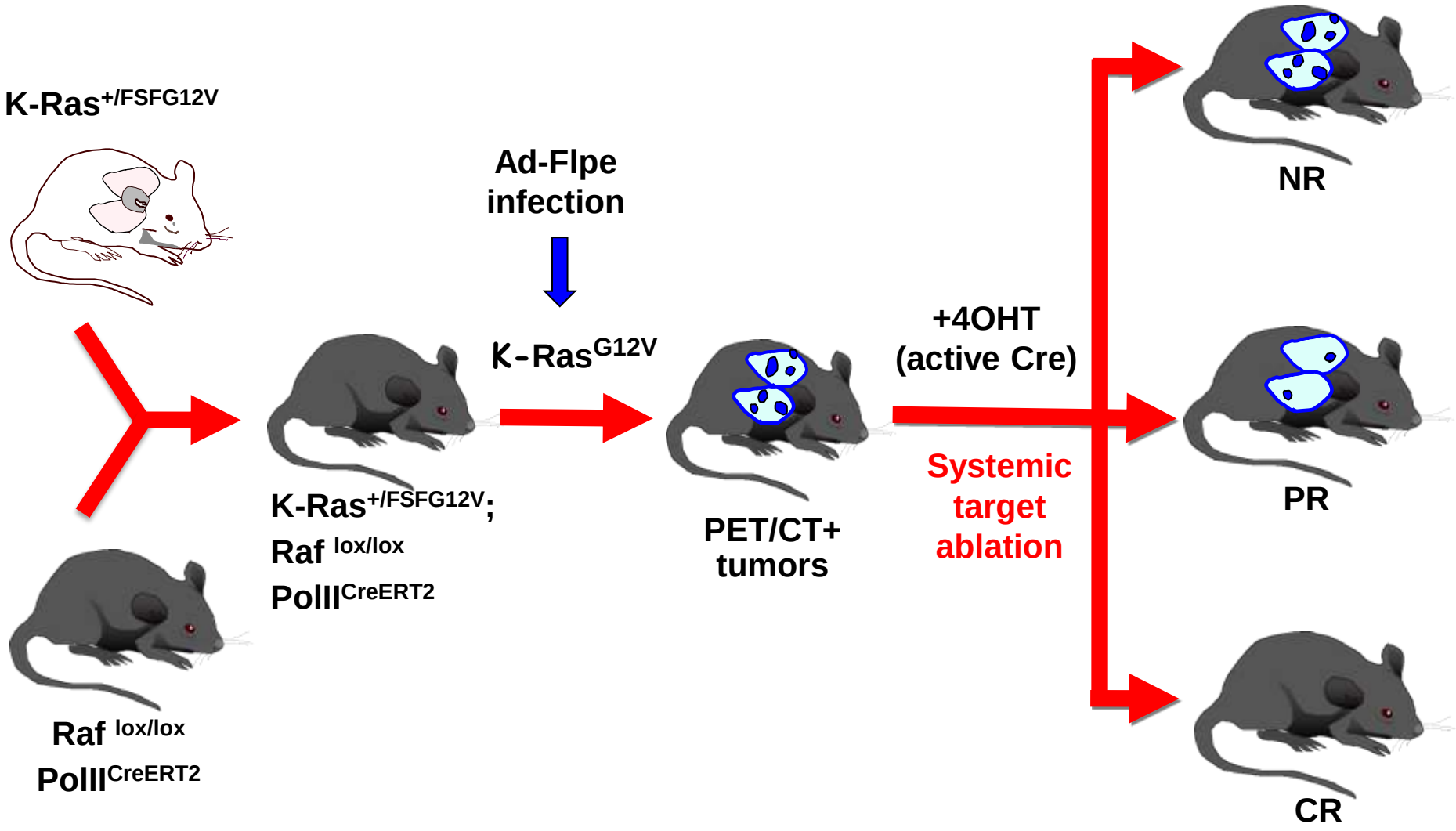
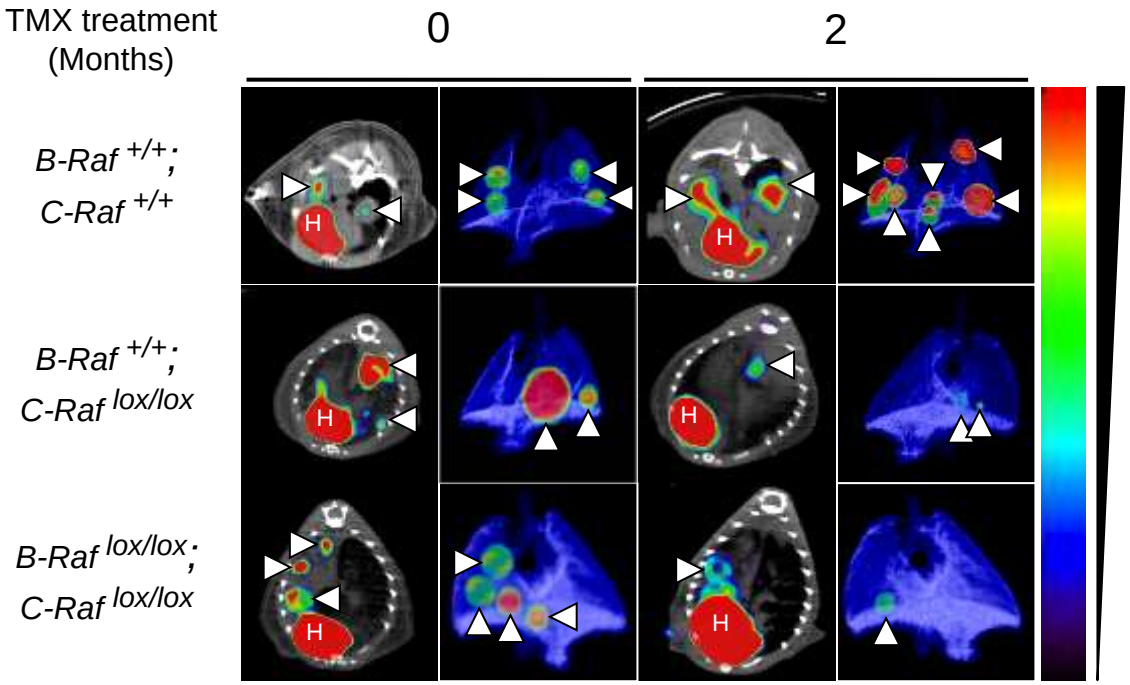
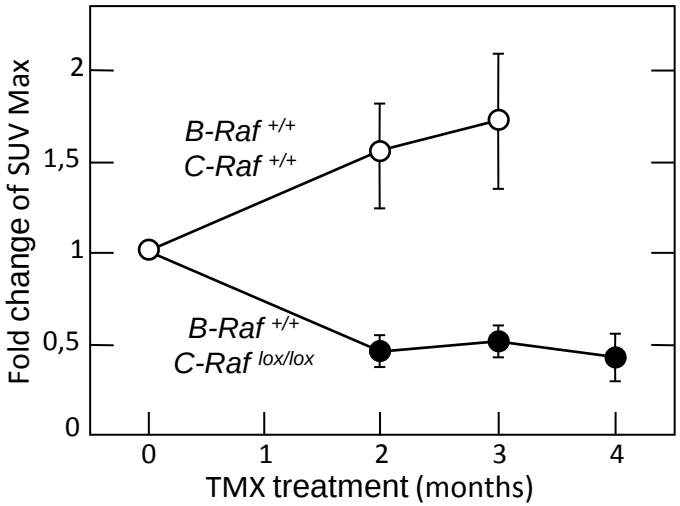


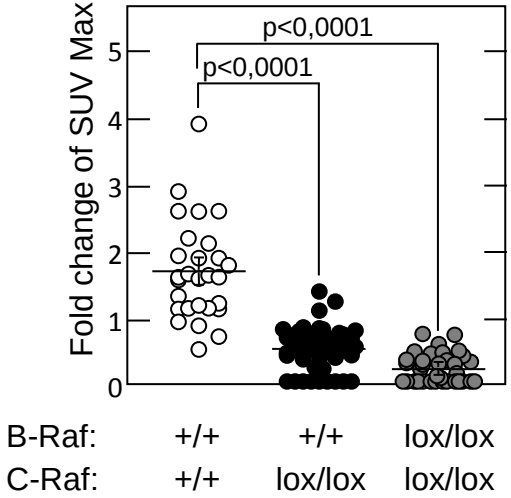
# *MAPK: Therapeutic approach*

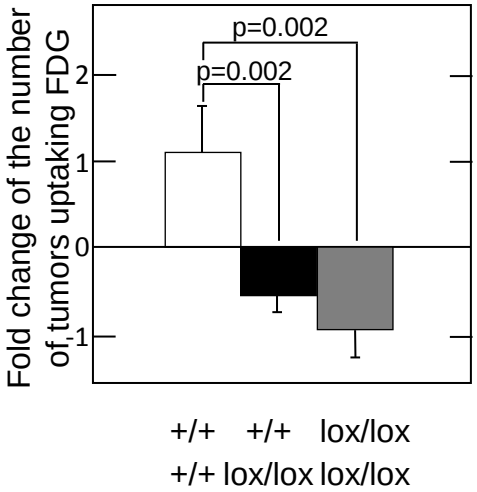


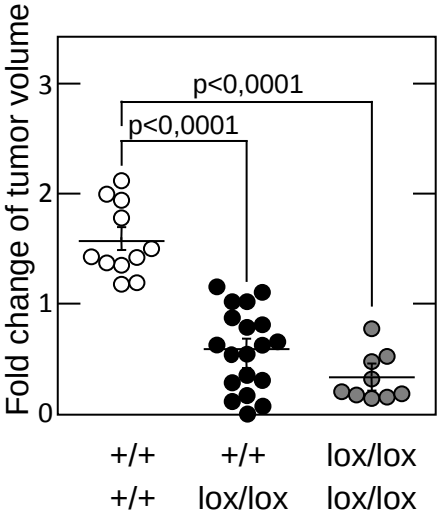
# MAPK: Therapeutic approach



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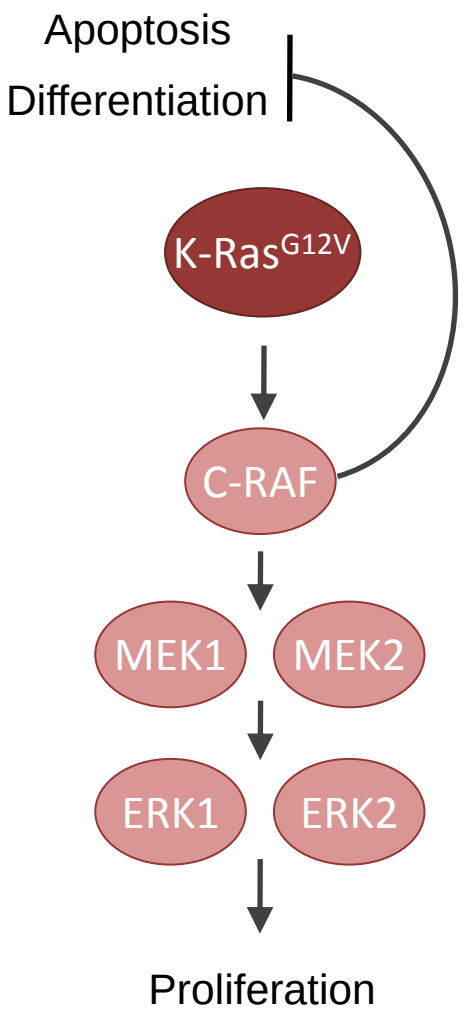
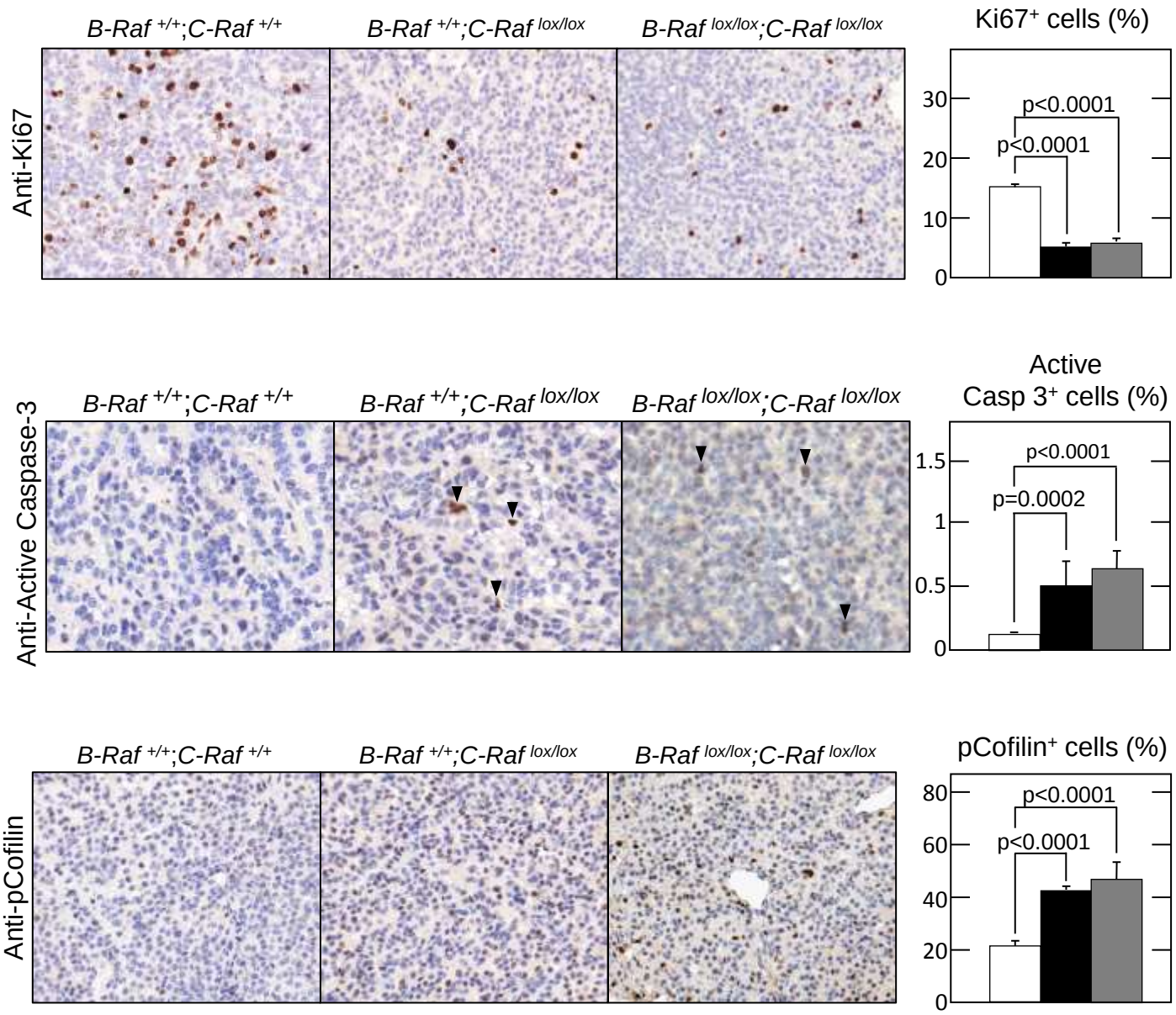
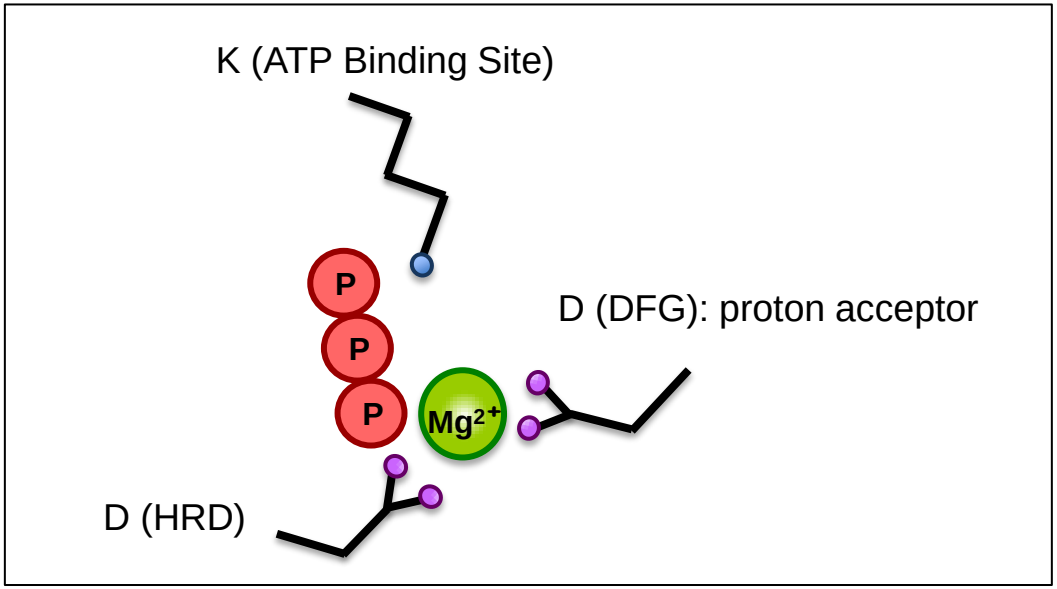
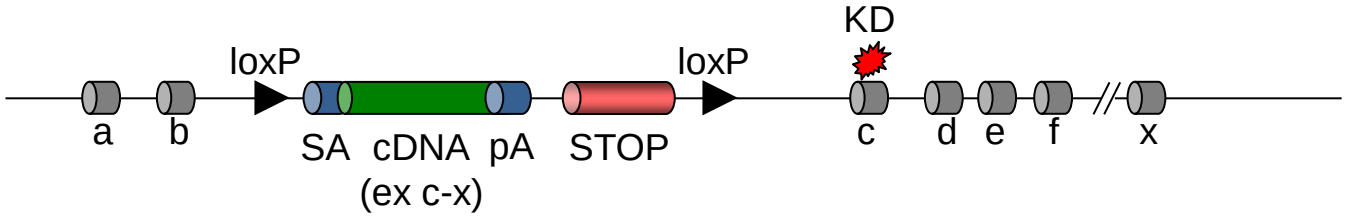
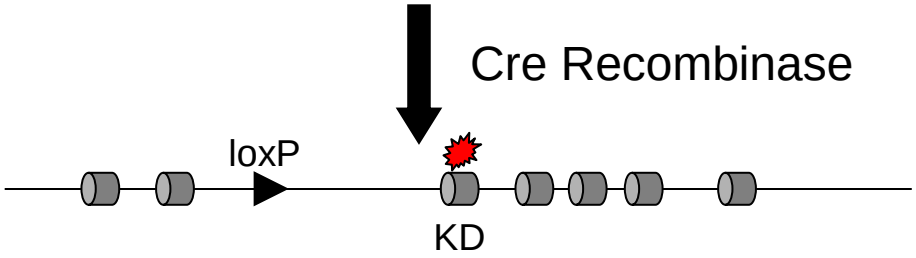


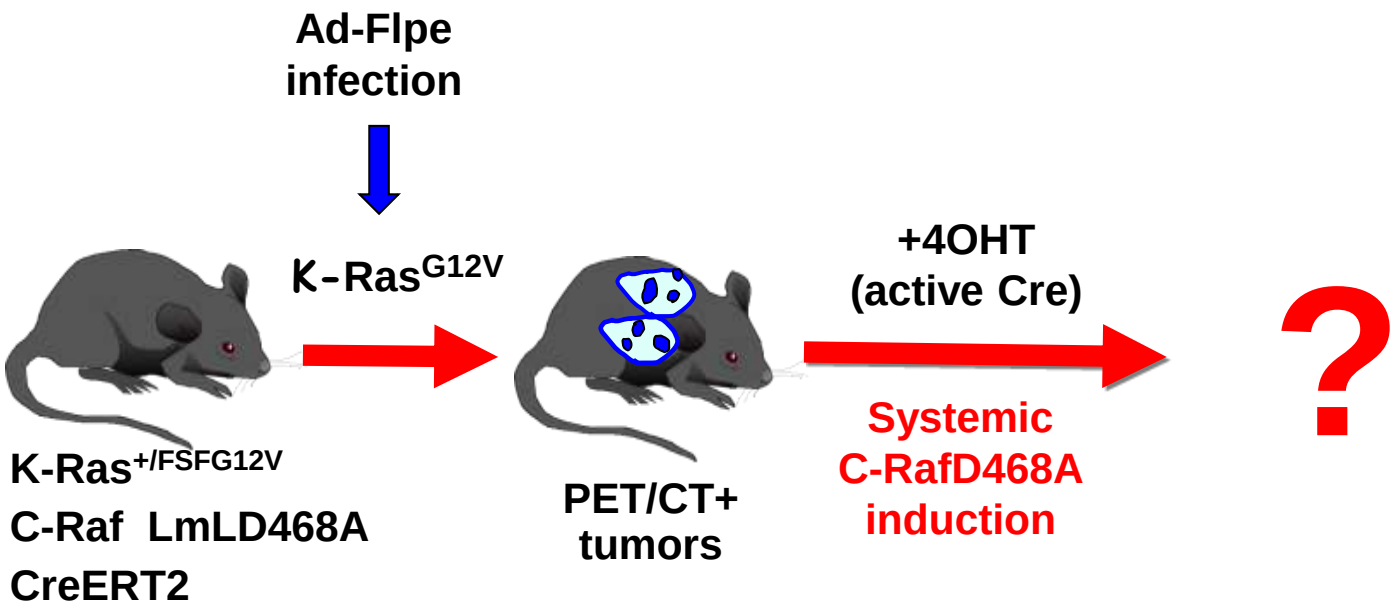




K (ATP Binding Site)
P
P
P
Mg2+
D (DFG): proton acceptor
D (HRD)

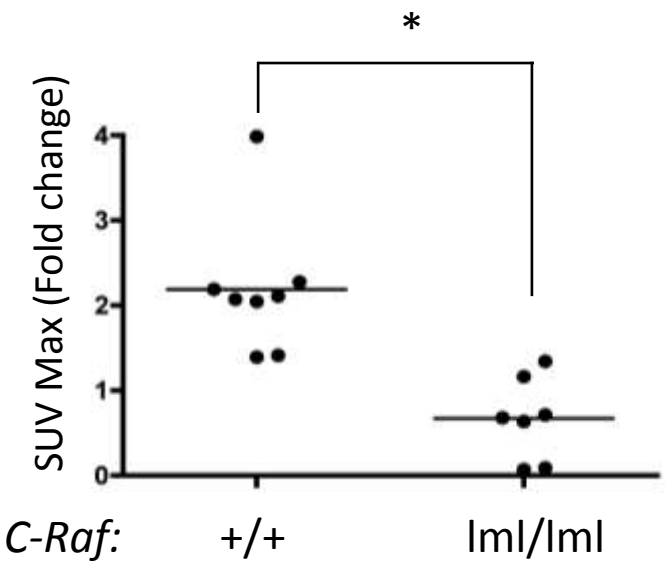

KD
loxP
loxP
a
b
SA
cDNA
pA
STOP
(ex c-x)
c
d
e
f
x

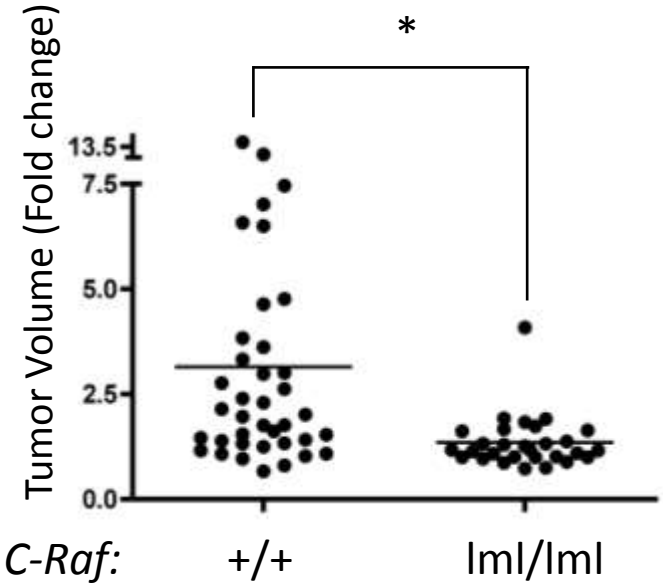

Cre Recombinase
loxP
KD

c-Raf D468A


Ad-Flpe
infection
K-Ras$^{G12V}$
+4OHT
(active Cre)
?
K-Ras$^{+/FSFG12V}$
C-Raf LmLD468A
CreERT2
PET/CT+
tumors
Systemic
C-RafD468A
induction

8 weeks Tamoxifen diet




*C-Raf* $^{LmLD468A/LmLD468A}$;*Tg.hUBC-CreERT2* $^{+/T}$ show no phenotype after 6 months of Tamoxifen diet

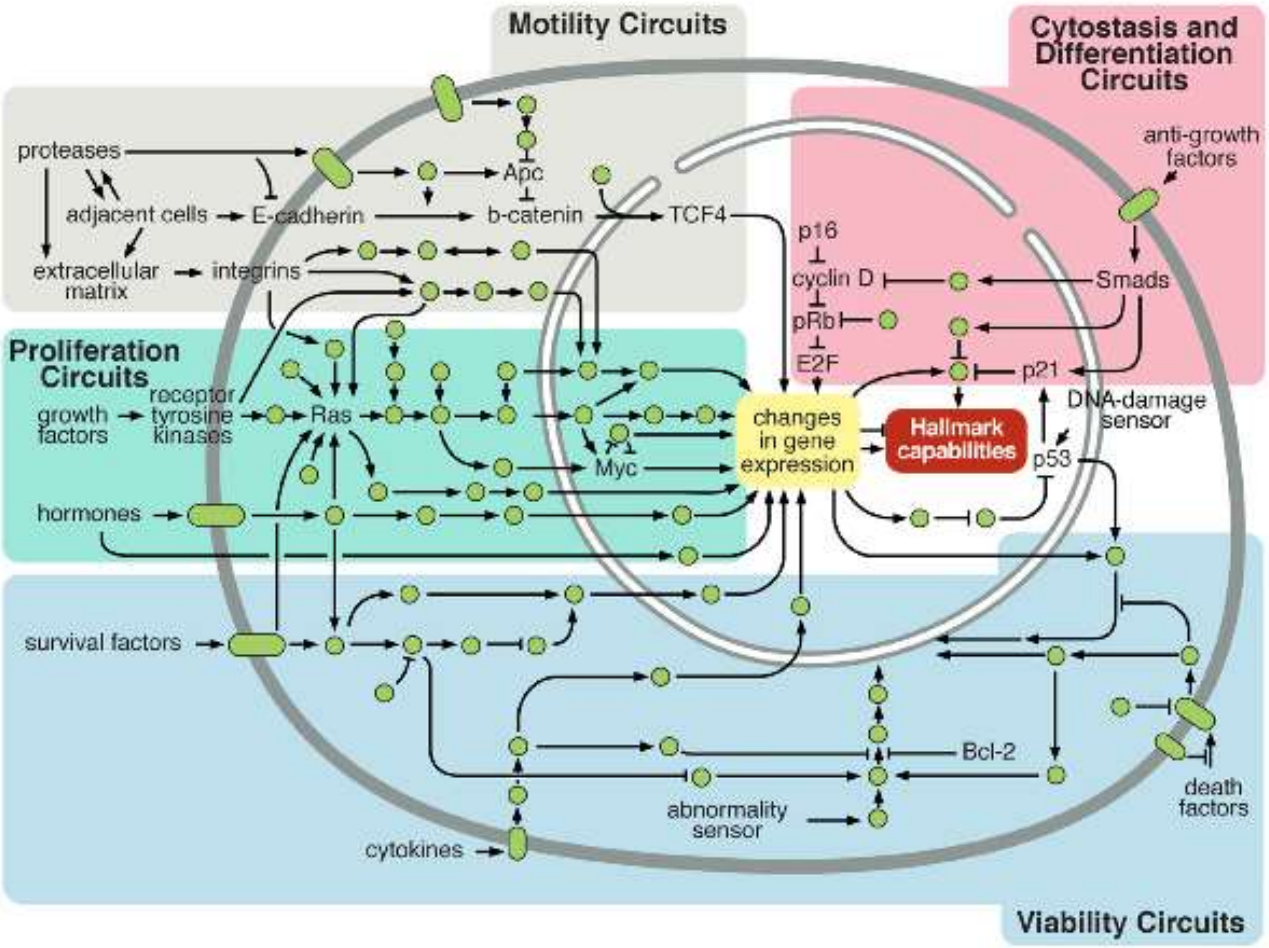
## *Conclussions (I)*

- Overall MAPK signalling is essential for mouse homeostasis and individual mediators are functionally redundant; their function is specialized in K-Ras$^{G12V}$-driven NSCLC.

- C-Raf is a valuable therapeutic target for K-Ras$^{G12V}$-driven NSCLC treatment with potentially low systemic toxicity.

- Inhibition of C-Raf catallytic activity induces regression of advanced K-Ras$^{G12V}$-driven NSCLC.

# *Combination therapy: choosing novel targets*




Hanahan & Weinberg, Cell 144 (2011)

# Combination therapy: choosing novel targets

nature biotechnology — ARTICLES

Assessing therapeutic responses in *Kras* mutant cancers using genetically engineered mouse models

Chronic cisplatin treatment promotes enhanced damage repair and tumor progression in a mouse model of lung cancer

*original article*

**Systemic Delivery of Tumor Suppressor microRNA Mimics Using a Neutral Lipid Emulsion Inhibits Lung Tumors in Mice**

Experimental Therapeutics, Molecular Targets, and Chemical Biology

**Dual Phosphoinositide 3-Kinase/Mammalian Target of Rapamycin Blockade Is an Effective Radiosensitizing Strategy for the Treatment of Non–Small Cell Lung Cancer Harboring *K-RAS* Mutations**

Cancer Cell — Article — Cell Press

**A Synthetic Lethal Interaction between K-Ras Oncogenes and Cdk4 Unveils a Therapeutic Strategy for Non-small Cell Lung Carcinoma**

PNAS

**Suppression of non-small cell lung tumor development by the *let-7* microRNA family**

Madhu S. Kumar*, Stefan J. Erkeland†, Ryan E. Pester*, Cindy Y. Chen*, Margaret S. Ebert*, Phillip A. Sharp*‡ and Tyler Jacks*‡§

*Center for Cancer Research and ‡Department of Biology, Howard Hughes Medical Institute, Massachusetts Institute of Technology, Cambridg and †Department of Hematology, Erasmus University Medical Center, 3015 GE, Rotterdam, The Netherlands

Contributed by Phillip A. Sharp, December 31, 2007 (sent for review December 4, 2007)

**Cancer Research** — AACR

**The Differential Effects of Mutant *p53* Alleles on Advanced Murine Lung Cancer**

Research Article

**Inhibition of Mammalian Target of Rapamycin Reverses Alveolar Epithelial Neoplasia Induced by Oncogenic *K-ras***

**Cancer Resear**

**Mutations in BRAF and KRAS Converge on Activation of the Mitogen-Activated Protein Kinase Pathway in Lung Canc**

REVIEWS

TARGETING RAS SIGNALLING PATHWAYS IN CANCER THERAPY

nature — Vol 462 5 November 2009 doi:10.1038/nature08462

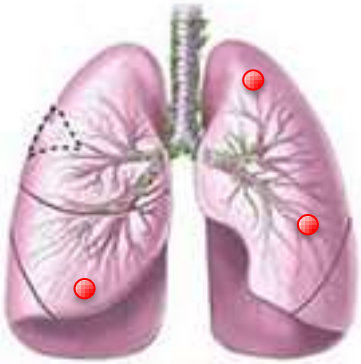
LETTERS

**Requirement for NF-κB signalling in a mouse model of lung adenocarcinoma**

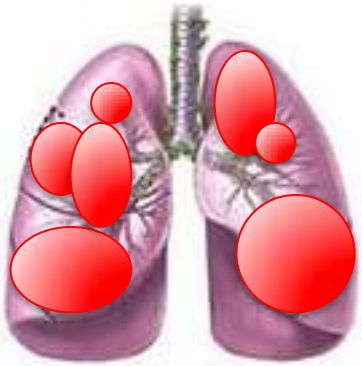
Cell Press — Cancer Cell — Article

**c-Raf, but Not B-Raf, Is Essential for Development of *K-Ras* Oncogene-Driven Non-Small Cell Lung Carcinoma**

**EARLY LESIONS**



**ADVANCED TUMORS**

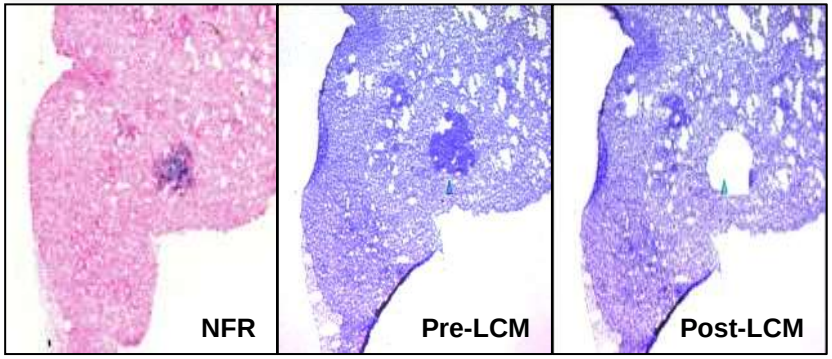


**Study EARLY lesions to:**

- **identify the nature of K-Ras-dependent essential mediators in NSCLC**
- **discover new druggable targets for K-Ras-driven NSCLC treatment**

# *Early stages in NSCLC progression*

**K-Ras$^{G12V}$ early NSCLC lesions**




(BIDMC, Boston)

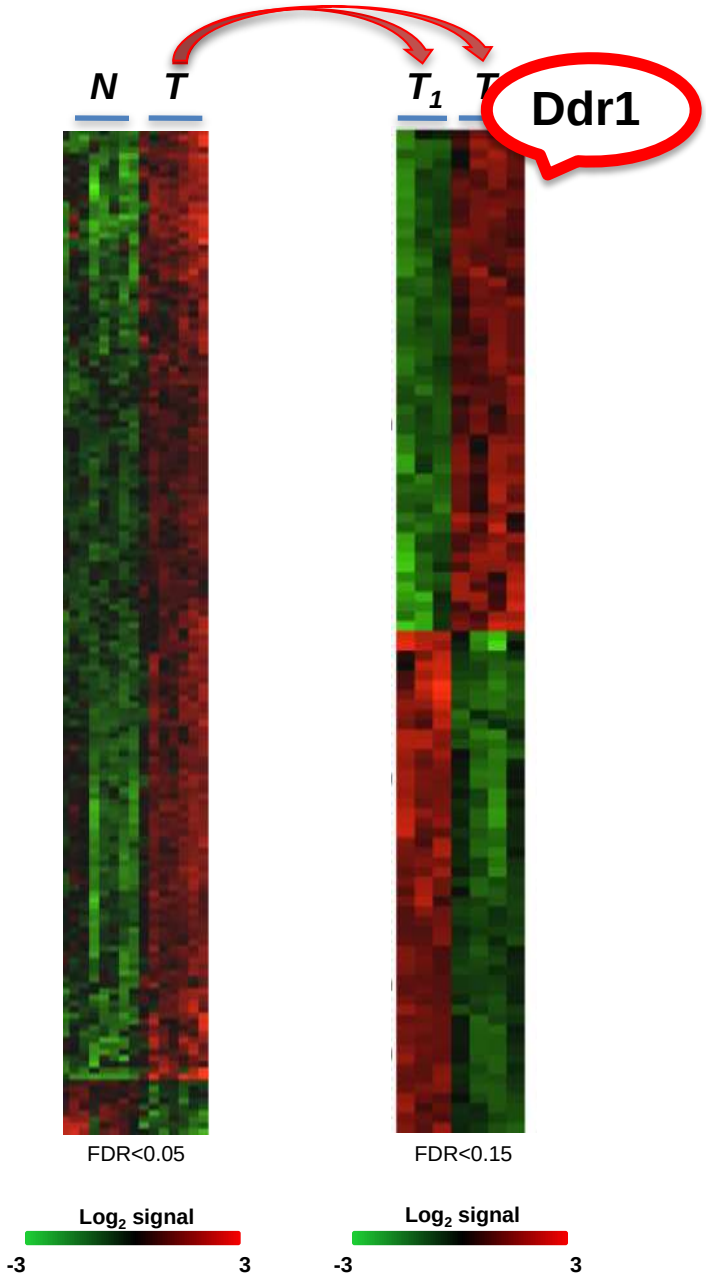
ARTICLES

nature genetics

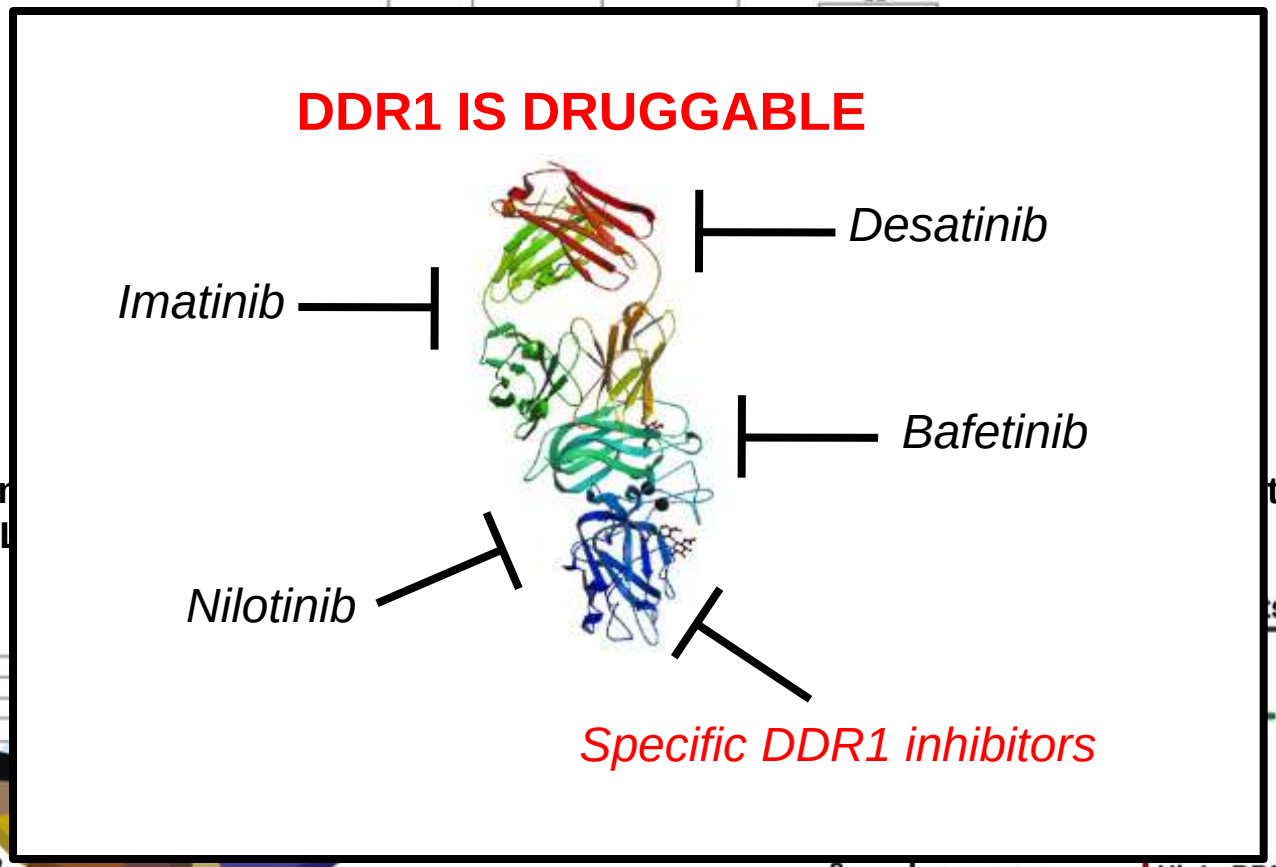
An oncogenic *KRAS2* expression signature identified by cross-species gene-expression analysis

Alejandro Sweet-Cordero[1], Sayan Mukherjee[3,4], Aravind Subramanian[3], Han You[1], Jeffrey J Roix[1], Christine Ladd-Acosta[3], Jill Mesirov[3], Todd R Golub[2,5] & Tyler Jacks[1,5]

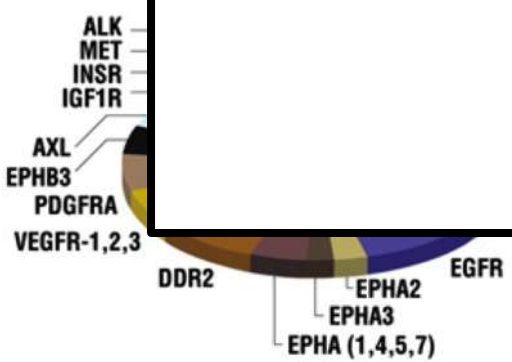
**p<0,0001**



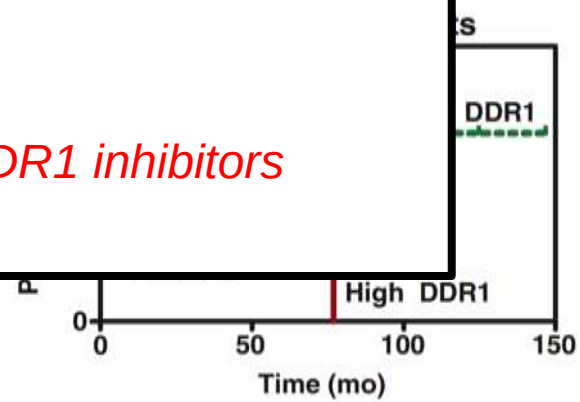
- **DDR1 is up-regulated in NSCLC compared to normal lung tissue, and in metastasis compared to primary tumors**




- **DDR1 is the r** … **tes with poor protein in NSCL**

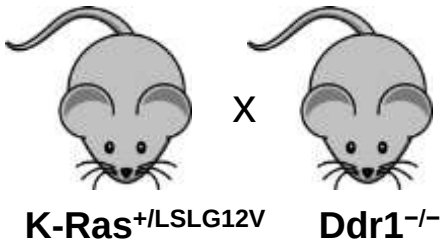


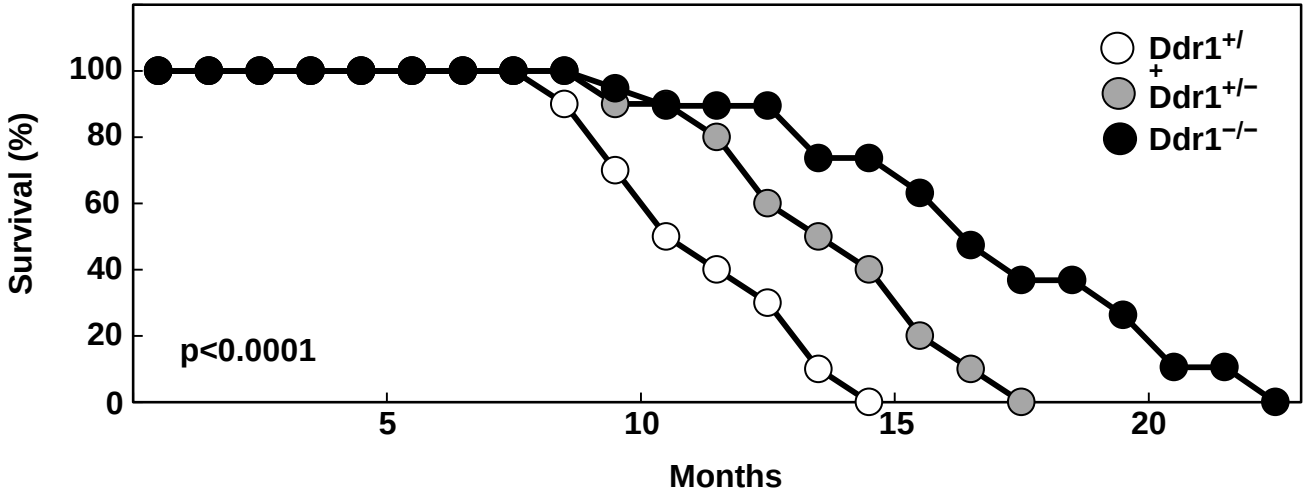

Rikova, K *et al.* (2007)

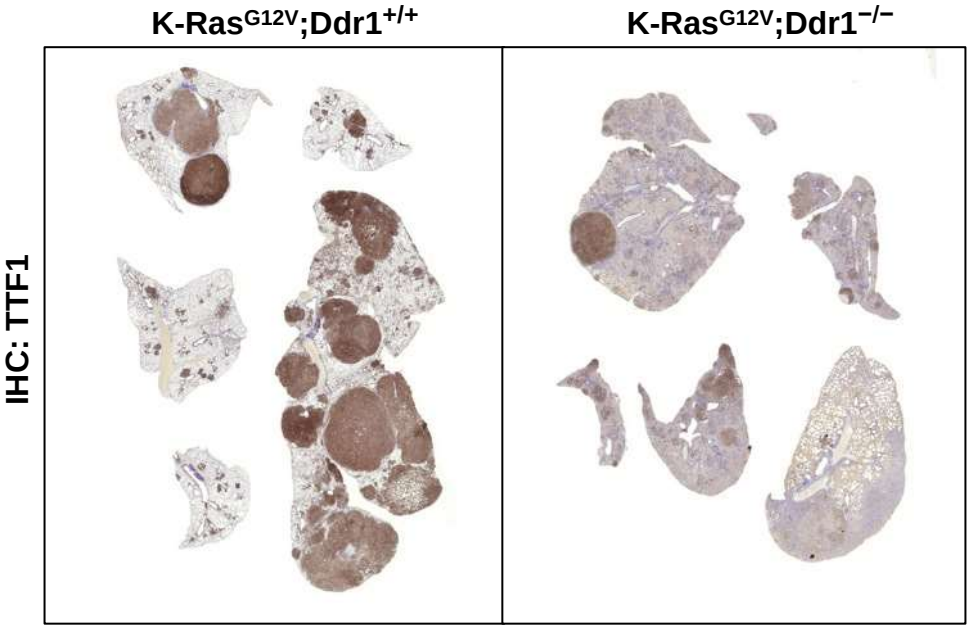


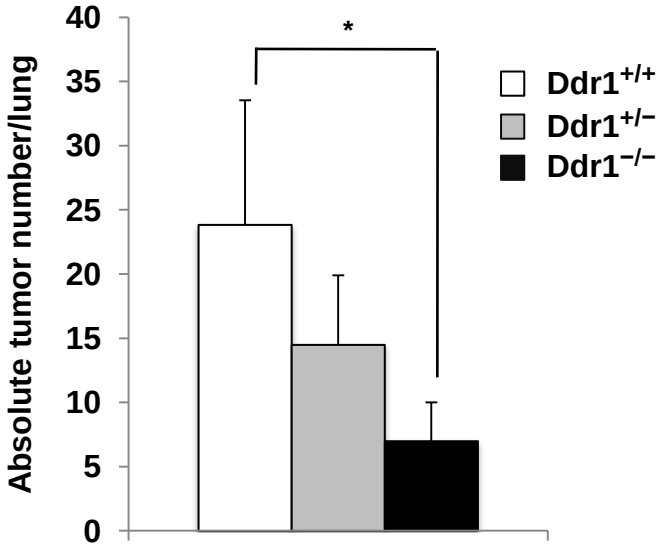

Valencia, K *et al*. (2012)

# *Ddr1 as a novel therapeutic target: genetic validation*

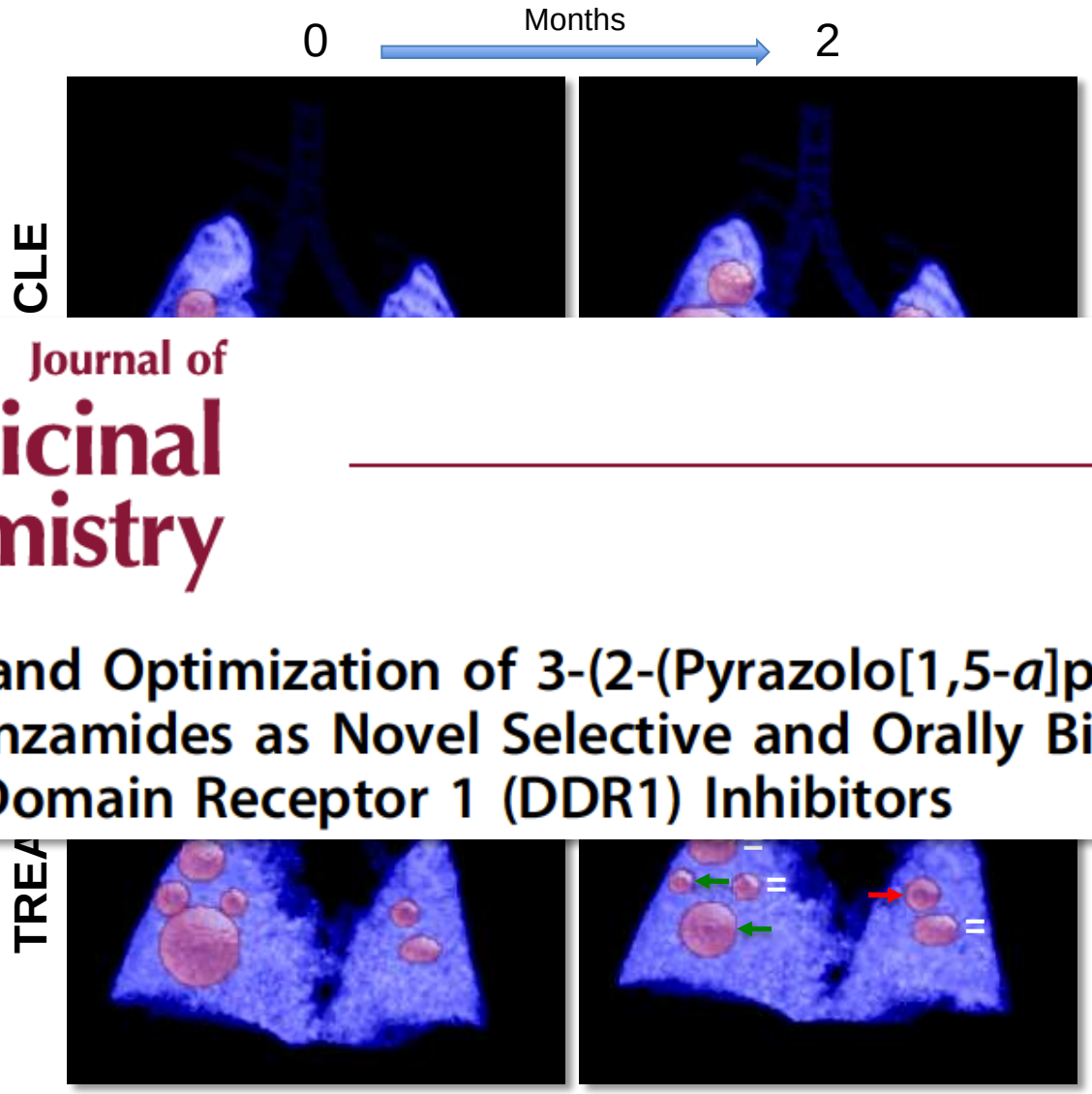


# *Ddr1 as a novel therapeutic target: pharmacologic validation*




Journal of Medicinal Chemistry

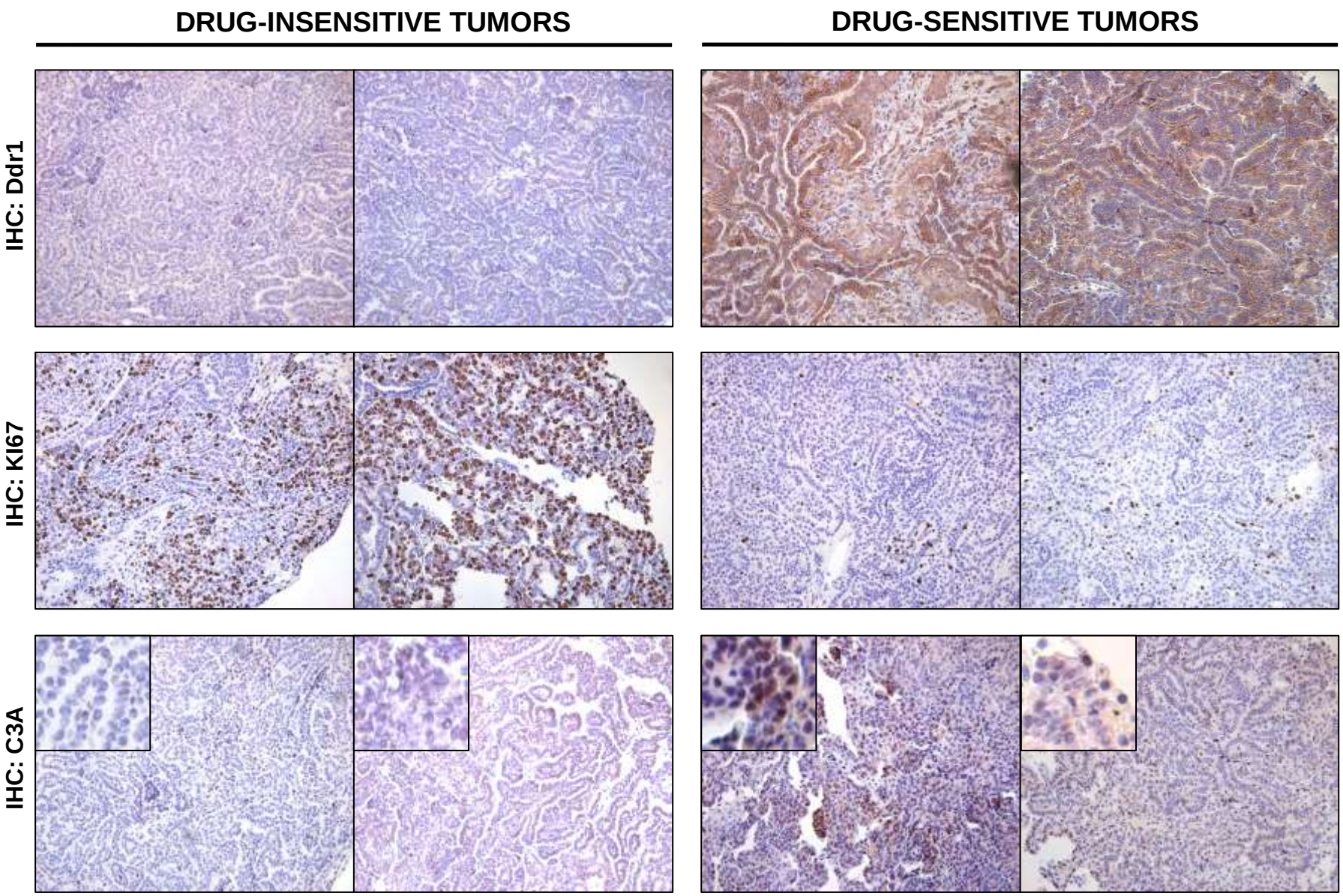
Article

pubs.acs.org/jmc

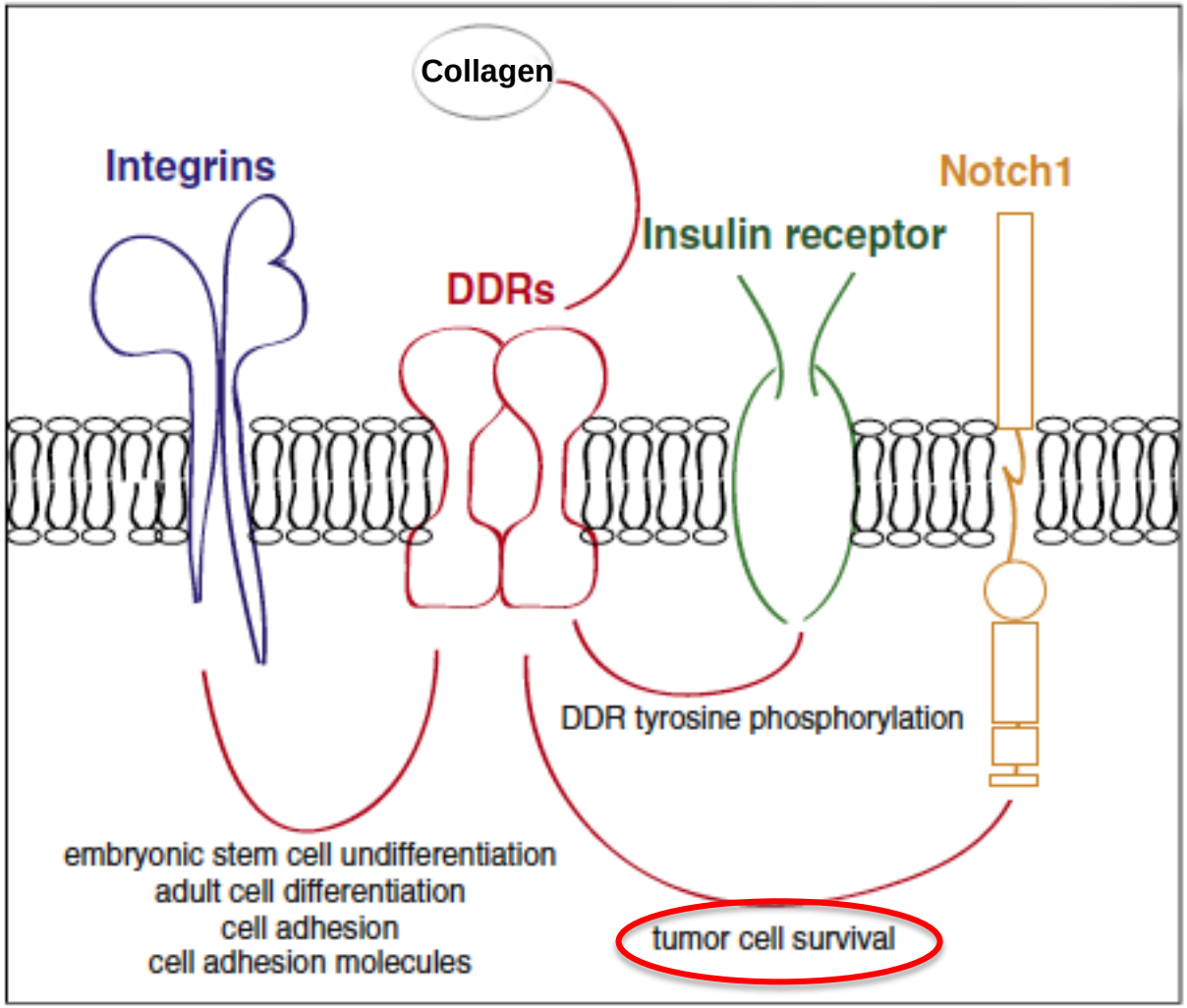
**Discovery and Optimization of 3-(2-(Pyrazolo[1,5-*a*]pyrimidin-6-yl)-ethynyl)benzamides as Novel Selective and Orally Bioavailable Discoidin Domain Receptor 1 (DDR1) Inhibitors**

*** 7rh inhibitor treatment, 50mg/kg, daily oral administration**

# *Ddr1 as a novel therapeutic target: pharmacologic validation*



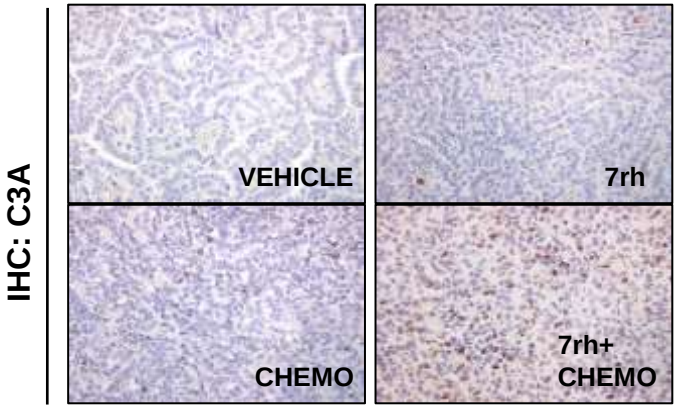
# *Ddr1 as a novel therapeutic target: in search of a mechanism*

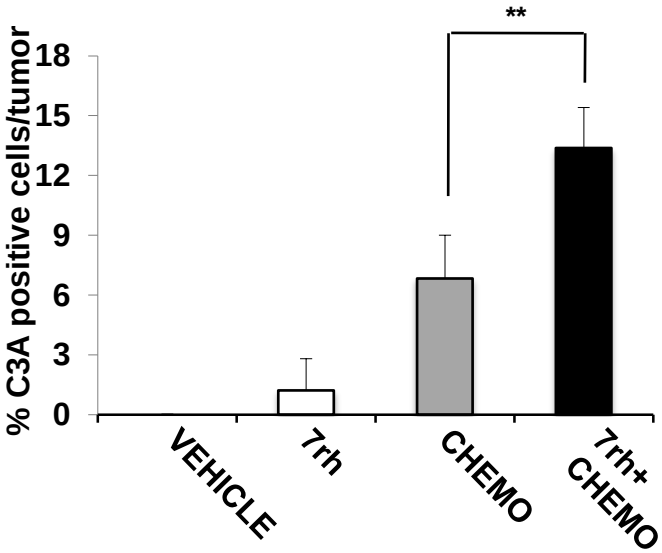



Adapted from Borza et al, *Matrix Biology* (2013)

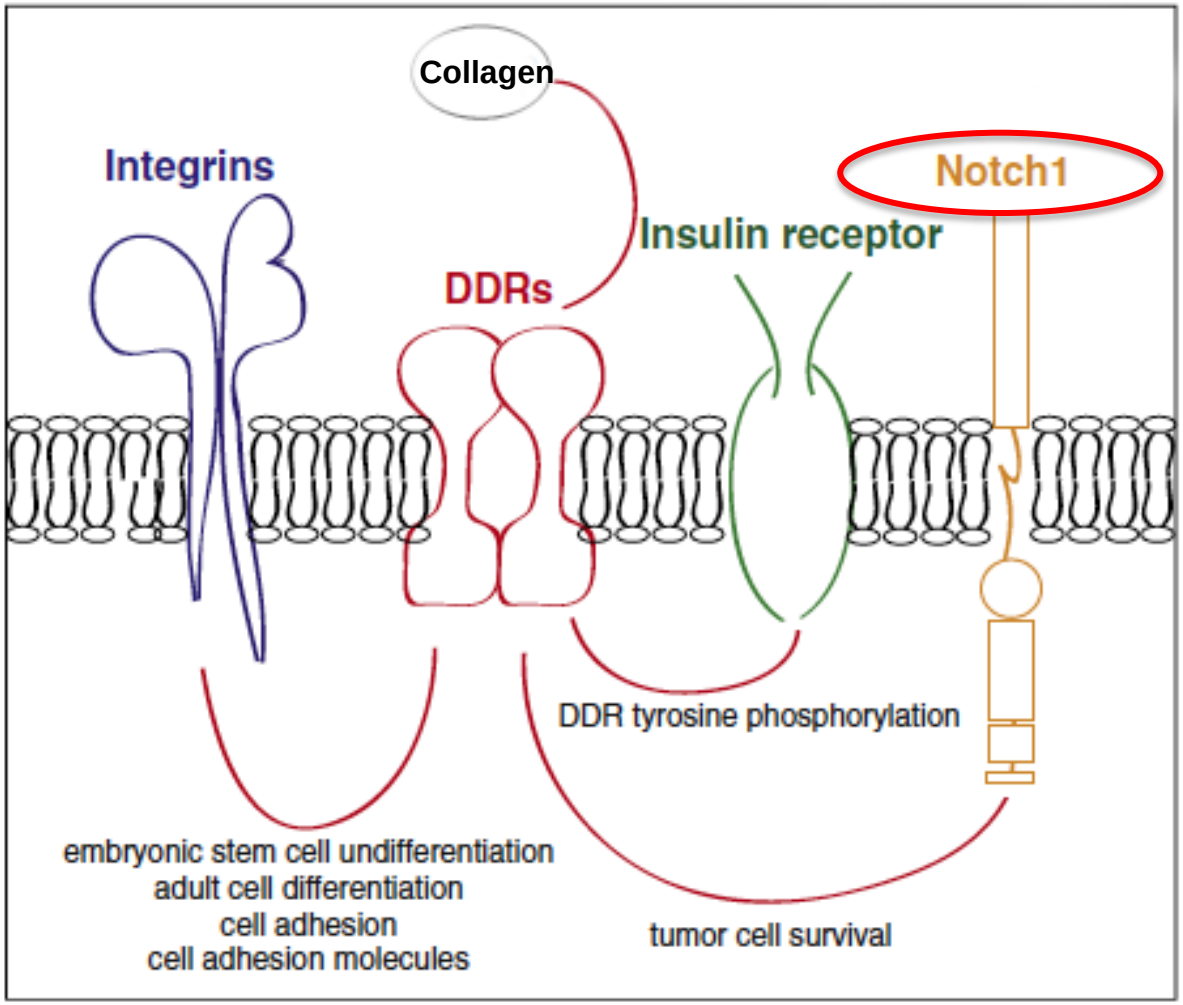
# *Ddr1 as a novel therapeutic target: in search of a mechanism*

**2-weeks treatment**



# *Ddr1 as a novel therapeutic target: in search of a mechanism*



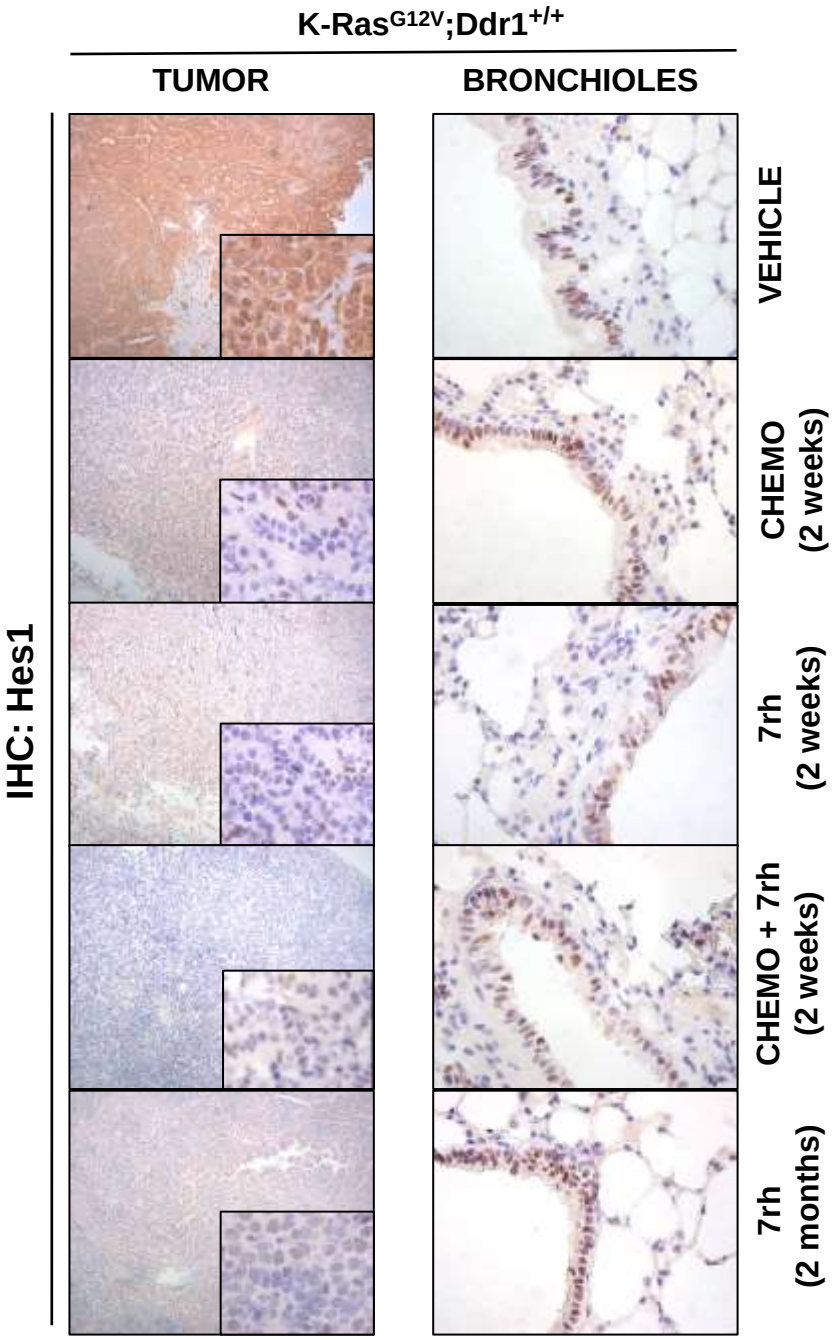

## DDR1 Receptor Tyrosine Kinase Promotes Prosurvival Pathway through Notch1 Activation*

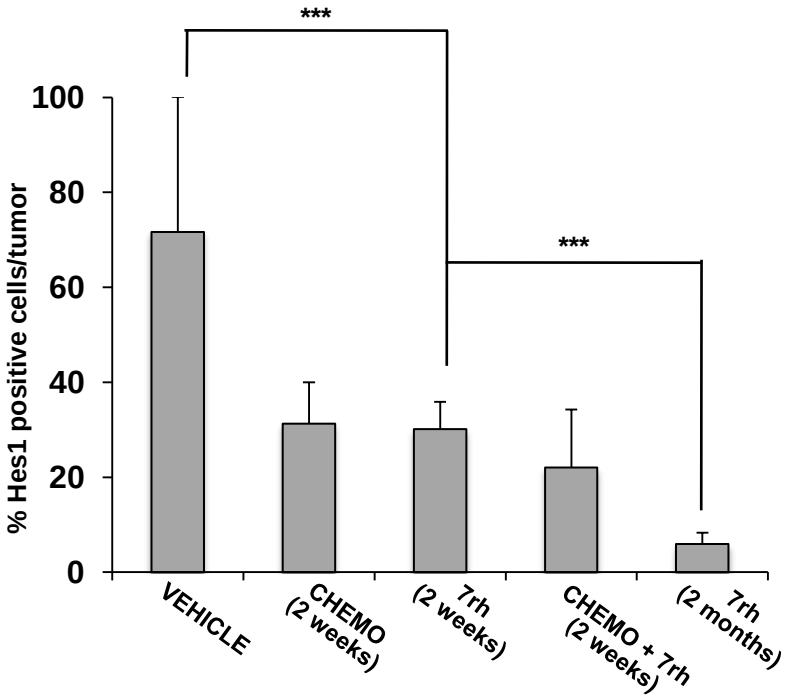
Received for publication, March 2, 2011 Published, JBC Papers in Press, March 13, 2011, DOI 10.1074/jbc.M111.236612

**Hyung-Gu Kim[‡], So-Young Hwang[‡], Stuart A. Aaronson[§], Anna Mandinova[‡], and Sam W. Lee[‡1]**

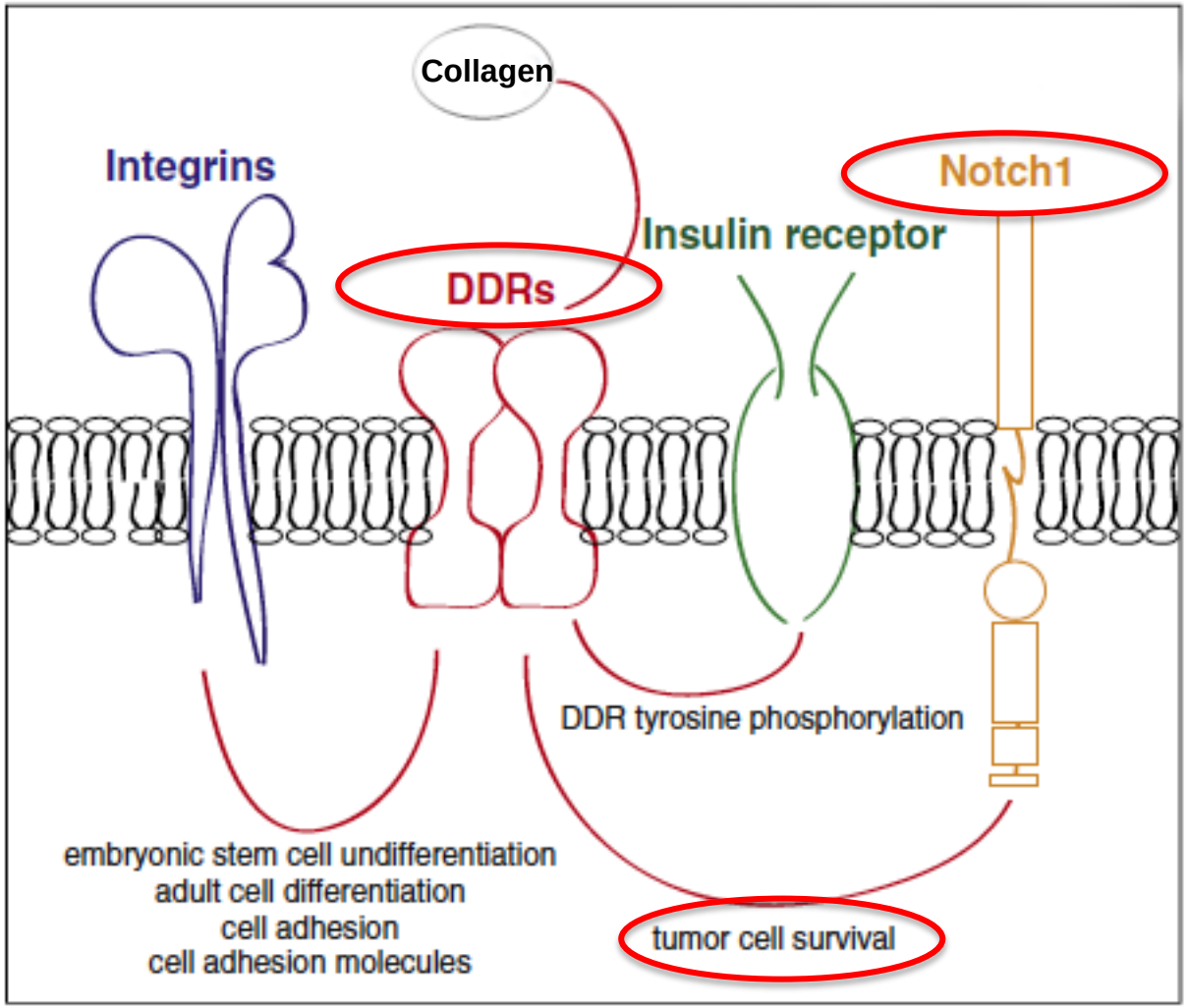
*From the [‡]Cutaneous Biology Research Center, Massachusetts General Hospital and Harvard Medical School, Charlestown, Massachusetts 02129 and the [§]Department of Oncological Sciences, Mount Sinai School of Medicine, New York, New York 10029*

# *Ddr1 as a novel therapeutic target: in search of a mechanism*



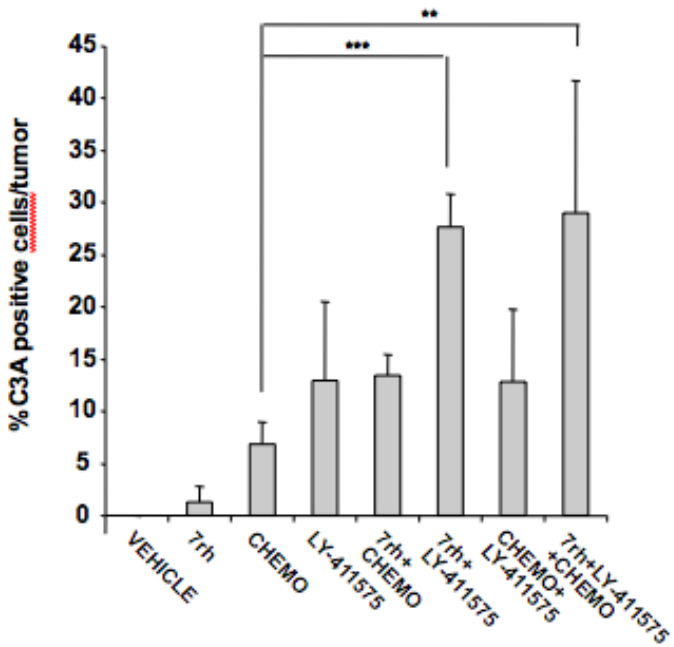



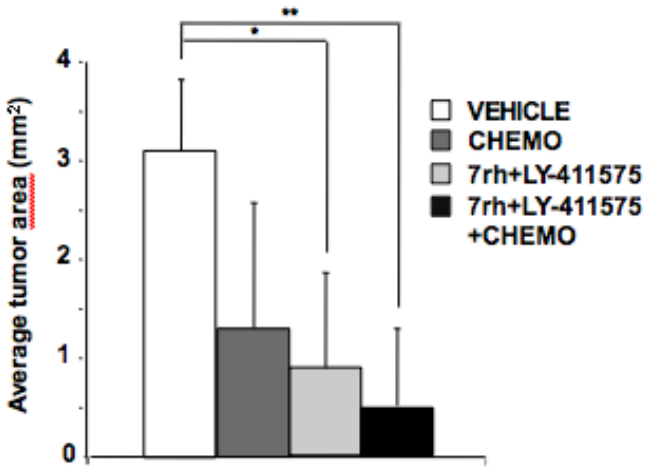
# *Ddr1 as a novel therapeutic target: in search of a mechanism*

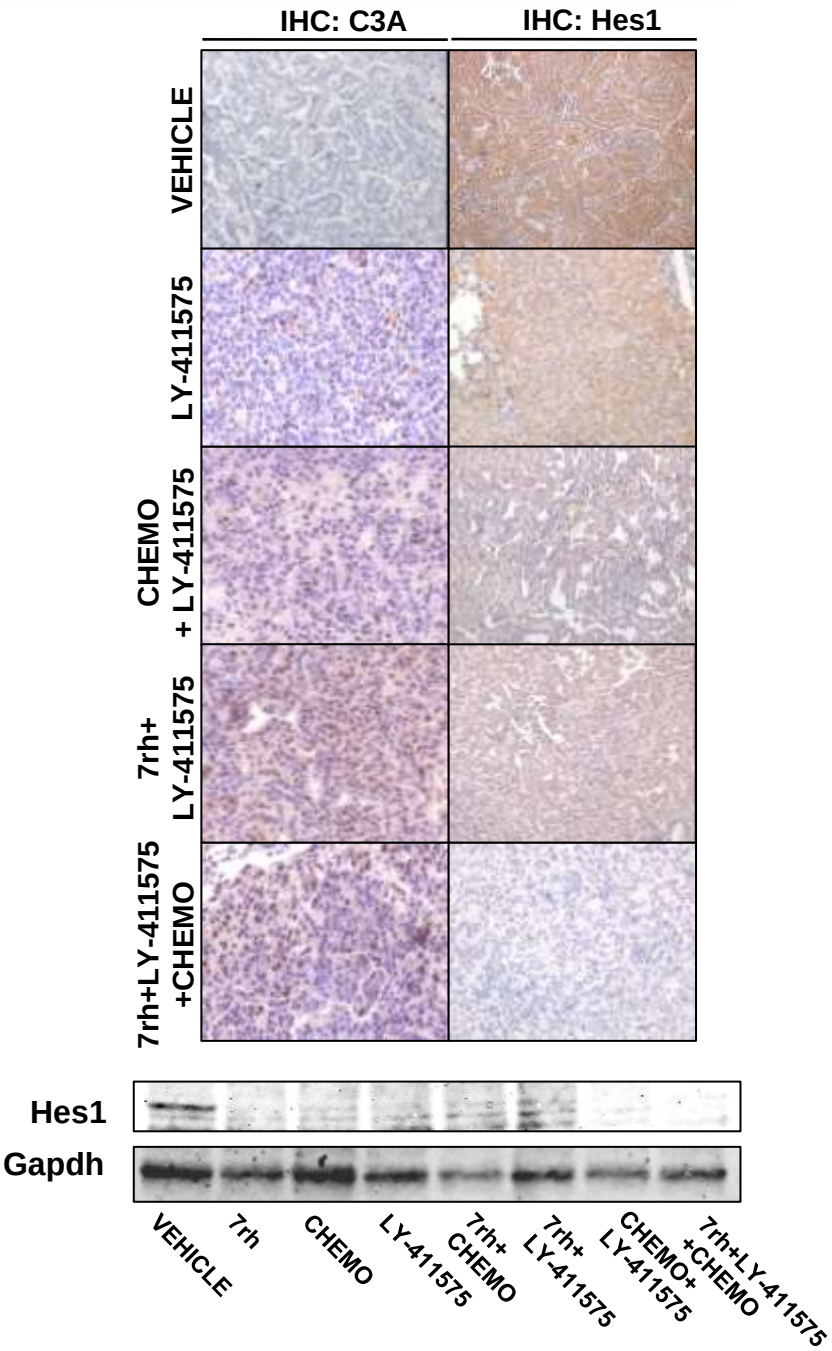



Adapted from Borza et al, *Matrix Biology* (2013)

# *Ddr1 as a novel therapeutic target: in search of a mechanism*

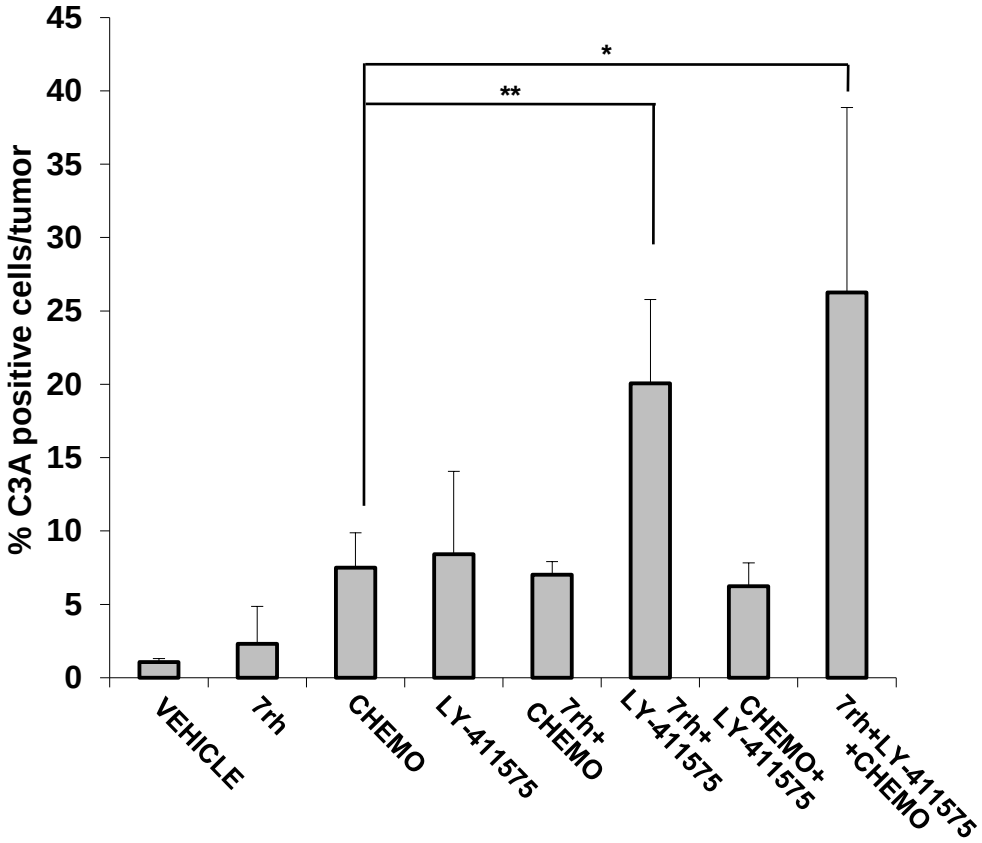


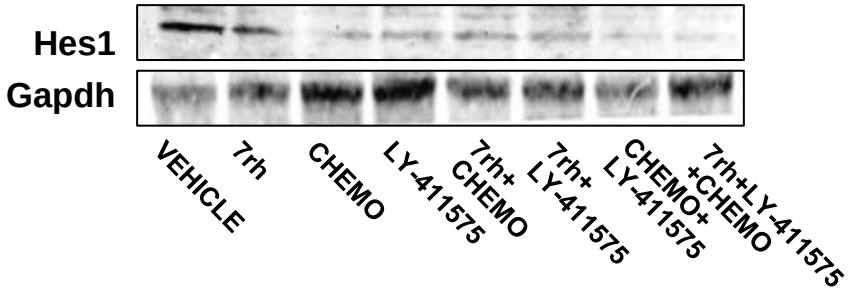



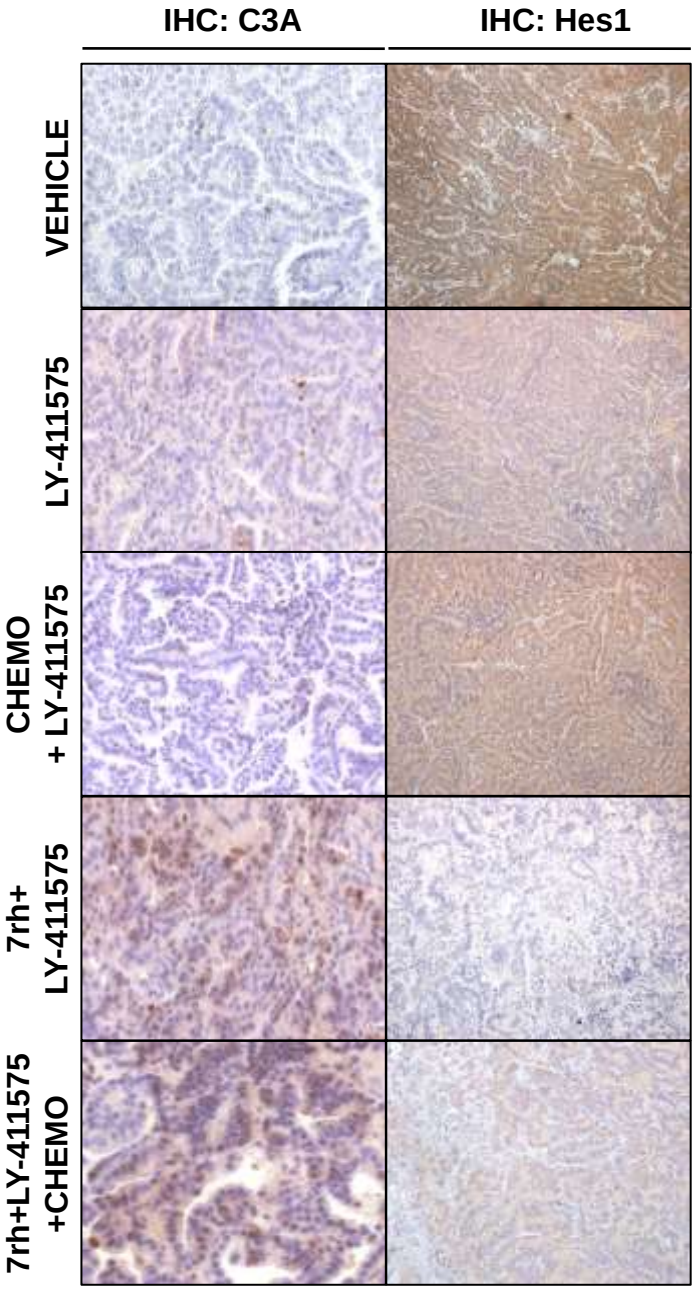



| | *p53*$^{+/+}$ | *p53*$^{lox/lox}$ |
|---|---|---|
| ***GENETIC DELETION OF Ddr1*** | | |
| • **Increase in overall survival** | ✔ | ✗ |
| • **Decrease in tumor number** | ✔ | ✗ |
| • **Decrease in tumor size** | ✔ | ✗ |
| • **Effect in tumor grading** | ✔ | ✗ |
| ***PHARMACOLOGICAL INHIBITION OF Ddr1*** | | |
| • **Efficacy of 7rh as single agent** | ✔ | ✗ |
| • **Efficacy of 7rh in combination with chemotherapy** | ✔ | ✗ |
| • **Efficacy of 7rh in combination with LY-411575** | ✔ | ✔ |

# *Ddr1 as a novel therapeutic target in aggressive NSCLC*



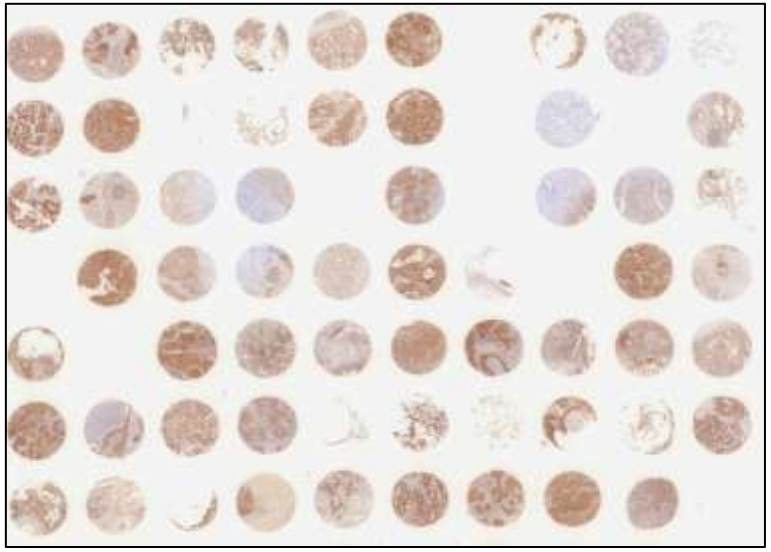
# *Is this applicable to human NSCLC?*

| Gene | K-RAS | DDR1 | HES1 |
|---|---|---|---|
| **K-RAS** | - - - | 0.011* | 0.025* |
| **DDR1** | | - - - | 0.012** |
| **HES1** | | | - - - |

n = 554 patients.
*Tendency towards co-occurrence (2<Odds ratio<10)
** Strong tendency towards co-occurrence (Odds ratio>10)

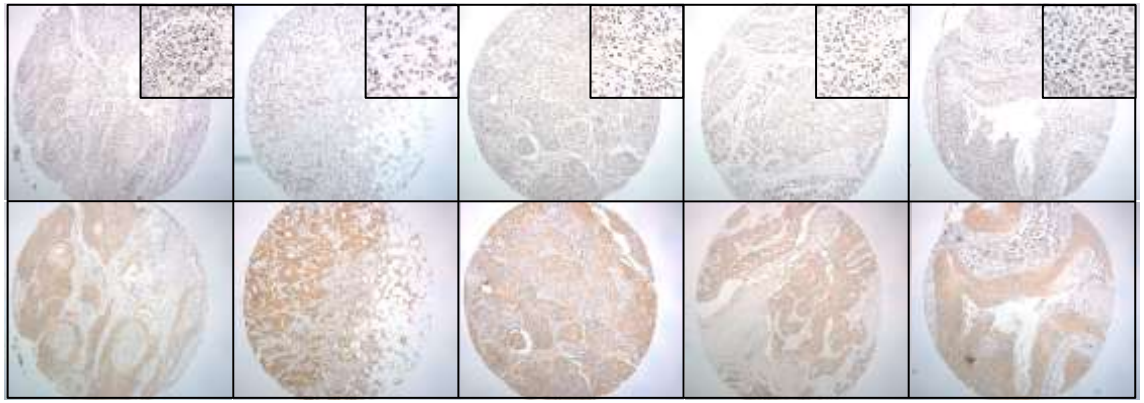
**IHC: DDR1**

**Human TMA**



**IHC: HES1**

**IHC: DDR1**



## *Conclussions (II)*

- ✓ Upon K-Ras$^{G12V}$ expression in lung, there are at least two different types of early lesions based on gene expression profiling (T1 and T2).

- ✓ T1 signature is closer to normal lung epithelial cells and could help to identify new tumor suppressor genes. T2 overlaps with advanced murine and human NSCLC signature, thus suggesting that the aggressiveness of lesions could be determined early during tumor onset.

- ✓ By an unbiased approach we identified Ddr1 as a valuable therapeutic target for K-Ras mutated NSCLC.

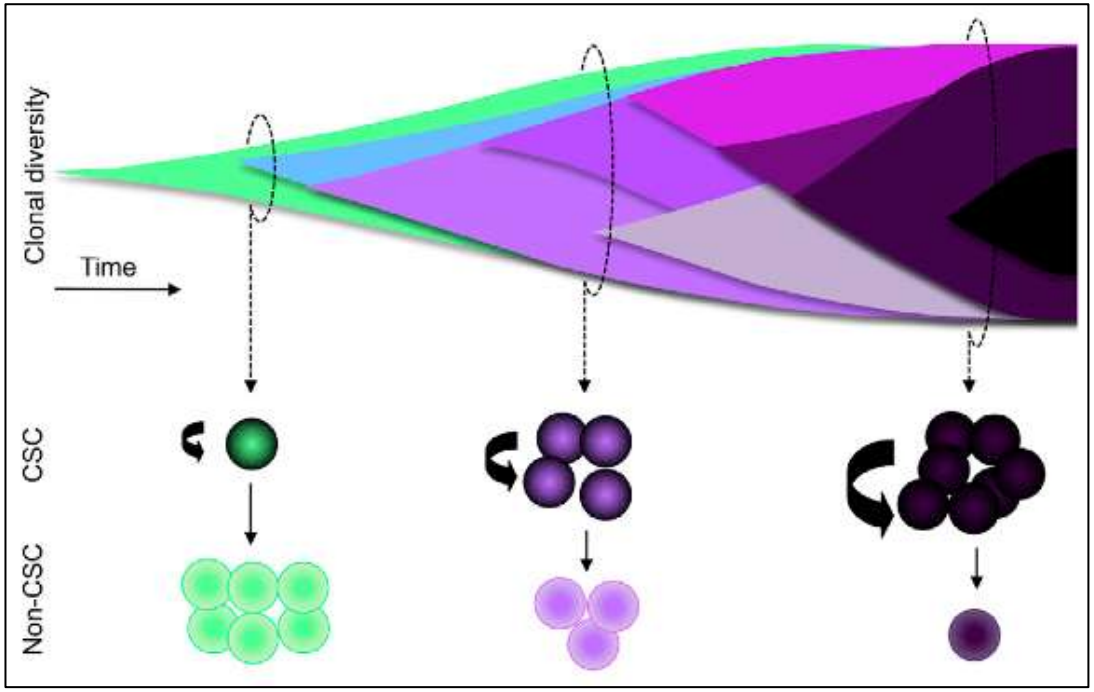
- ✓ Genetic ablation or pharmacological inhibition of Ddr1 impairs tumor growth in p53-proficient K-Ras$^{G12V}$ NSCLC.

- ✓ Combined inhibition of Ddr1 and Notch signaling efficiently triggers an apoptotic response even in p53-deficient K-Ras$^{G12V}$ NSCLC.

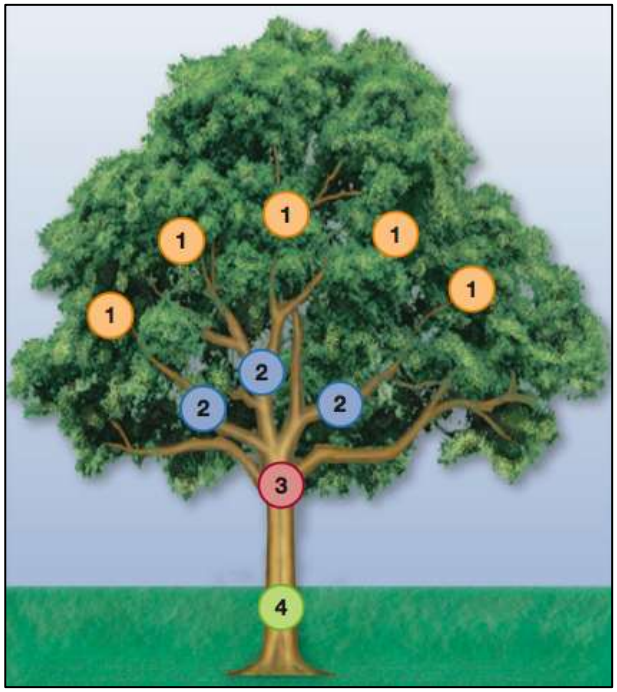
# *Take home message 1*

- There are >800 drugs targeting signaling pathways; finding the right drug (or combination of) for personalized treatment is challenging.

- Tumor evolution, heterogeneity and resistance.



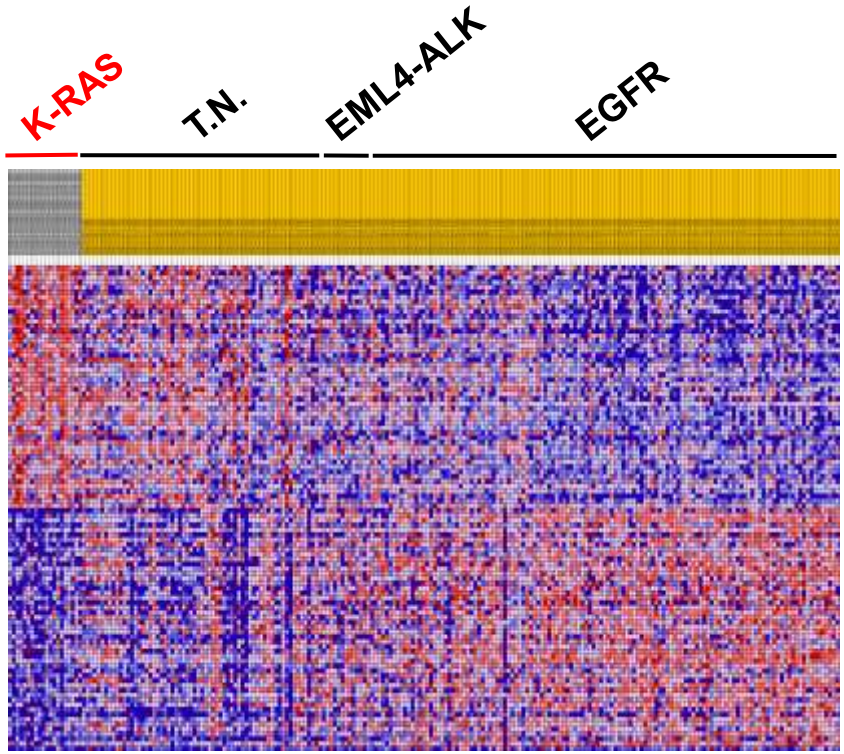

Kreso & Dick, Cell Stem Cell 2014



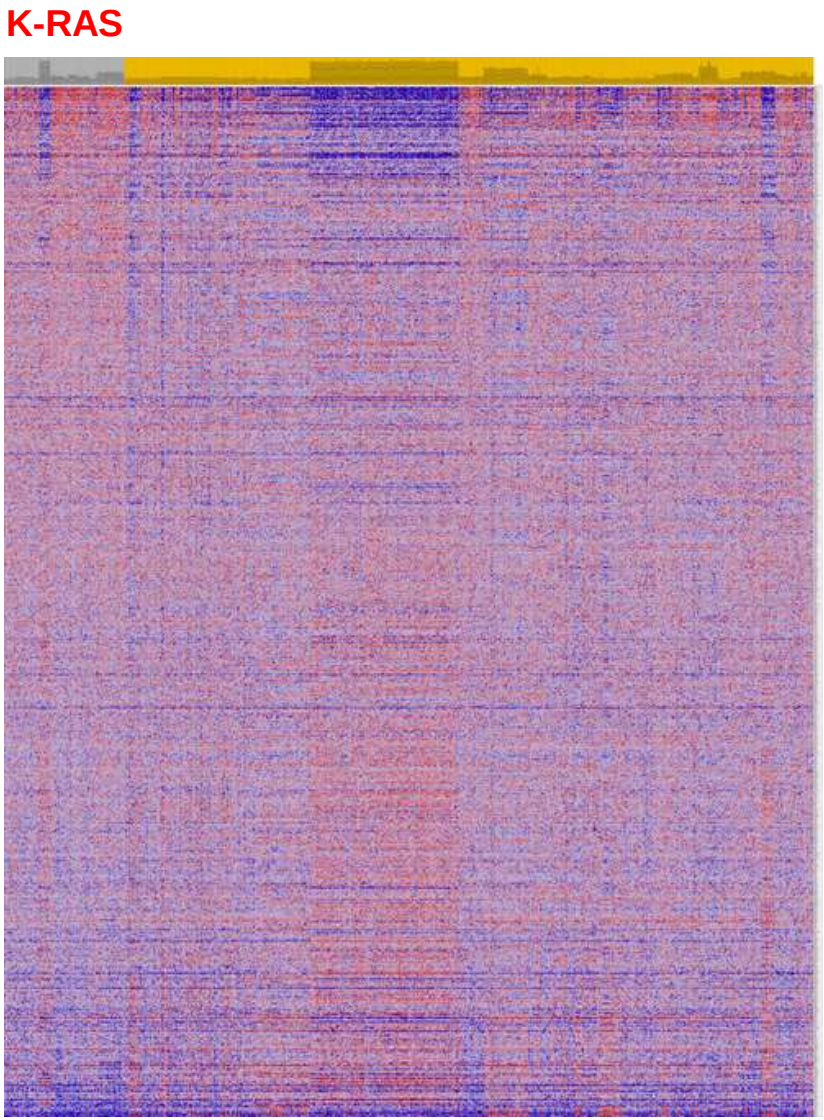

Swanton, Cancer Res 2012

*Take home message 1*

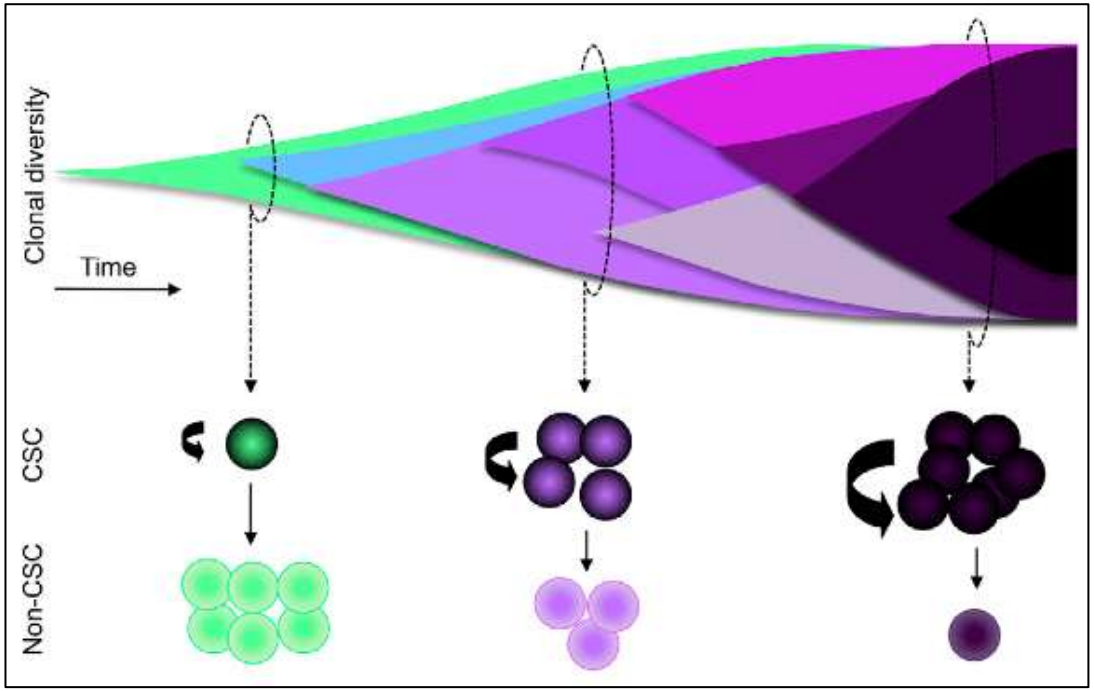
Human NSCLC



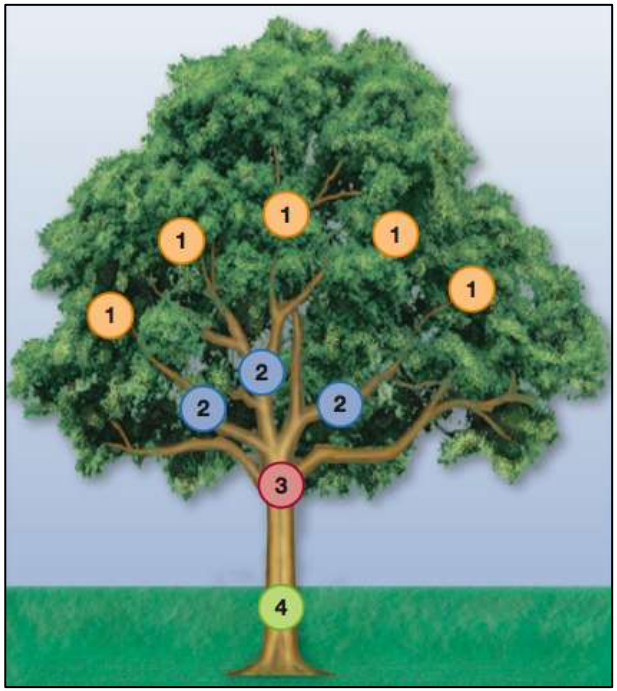

Cancer cell line encyclopedia



# *Take home message 1*

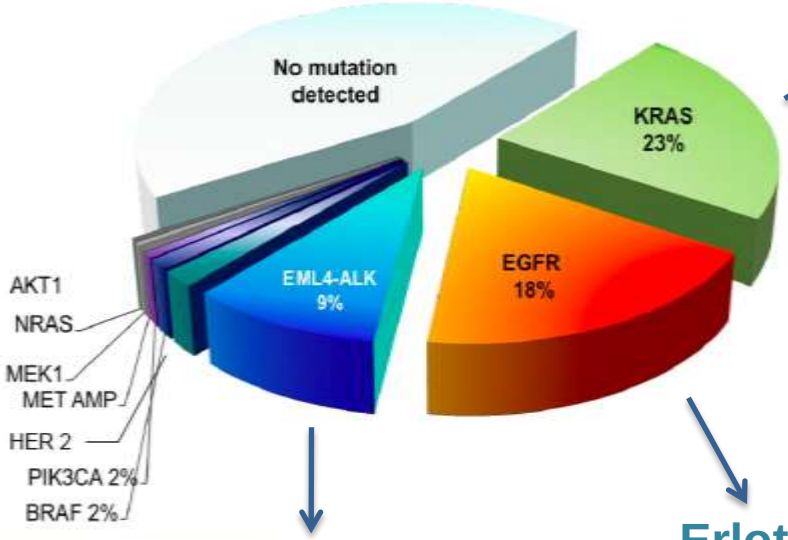



Kreso & Dick, Cell Stem Cell 2014




Swanton, Cancer Res 2012

# *Take home message 2*




**DDR1/NOTCH inhibitors**

**NO TARGETED THERAPY**

**Crizotinib**

**Erlotinib**

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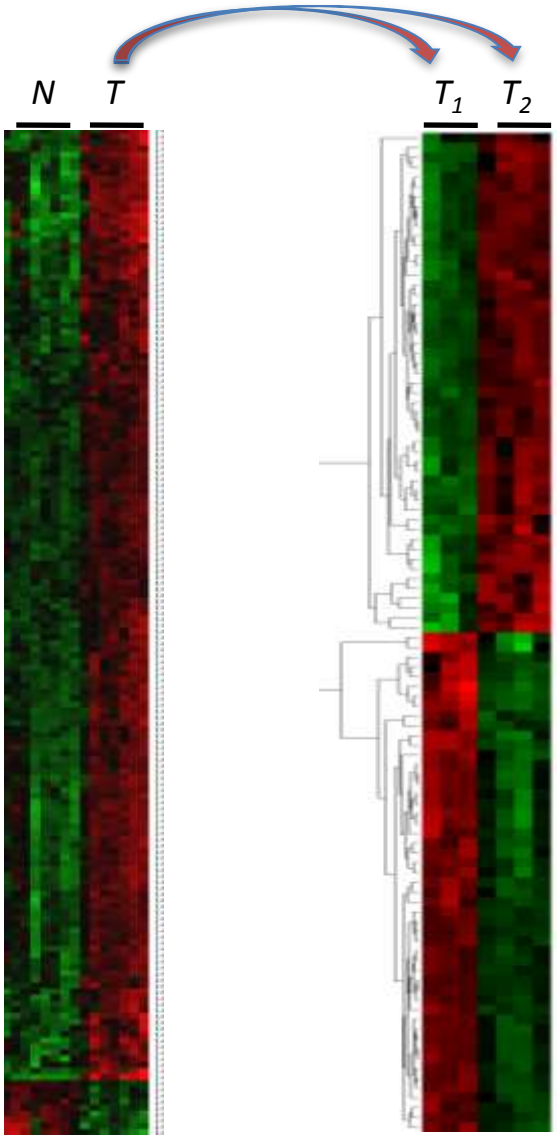
Rafael Blasco
Emilie Bousquet
Sarah Francoz
Patricia Nieto

**Collaborators:**
Ke Ding (GIBG, Guangzhou)
Pierre Dubus (Univ. Bordeaux)
Montserrat Sánchez-Céspedes (Idibell, Barcelona)
Manuel Serrano (CNIO)
Gonzalo Gómez (CNIO)

SEVENTH FRAMEWORK PROGRAMME
LUNGTARGET
GOBIERNO DE ESPAÑA
MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD
cnio Centro Nacional de Investigaciones Oncológicas

# Combination therapy: validating novel targets (II)

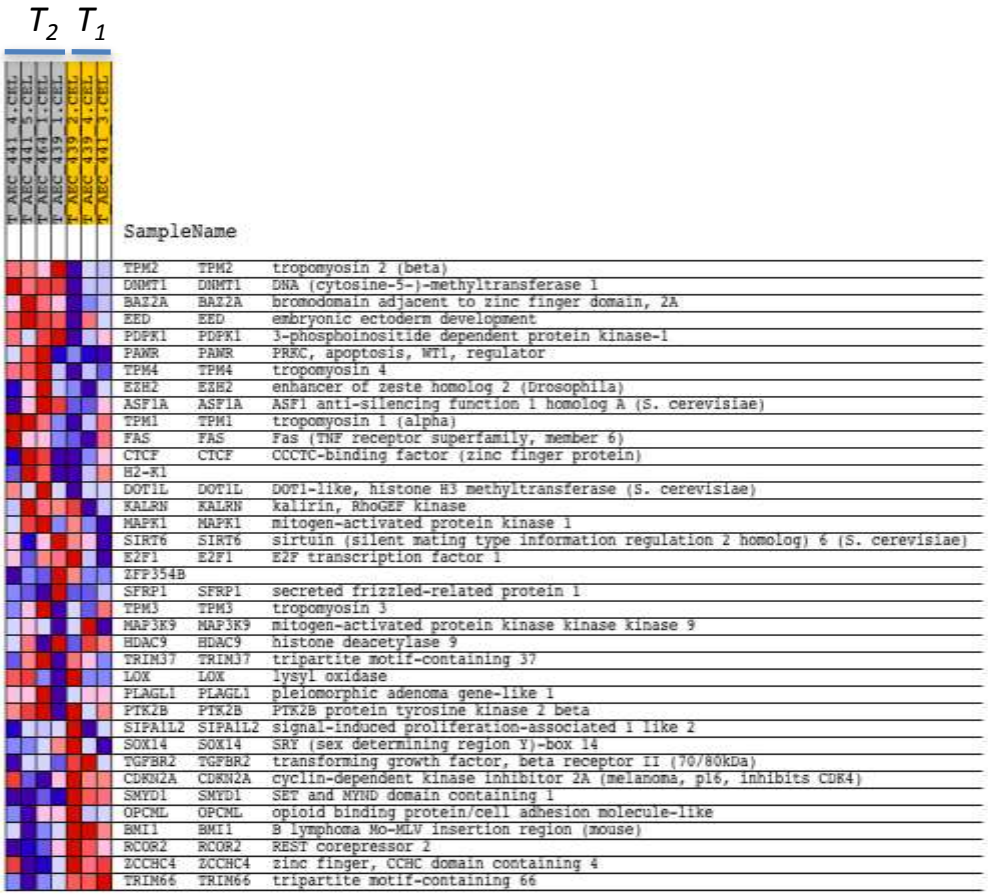



Vol 449 | 25 October 2007 | doi:10.1038/nature06251 nature

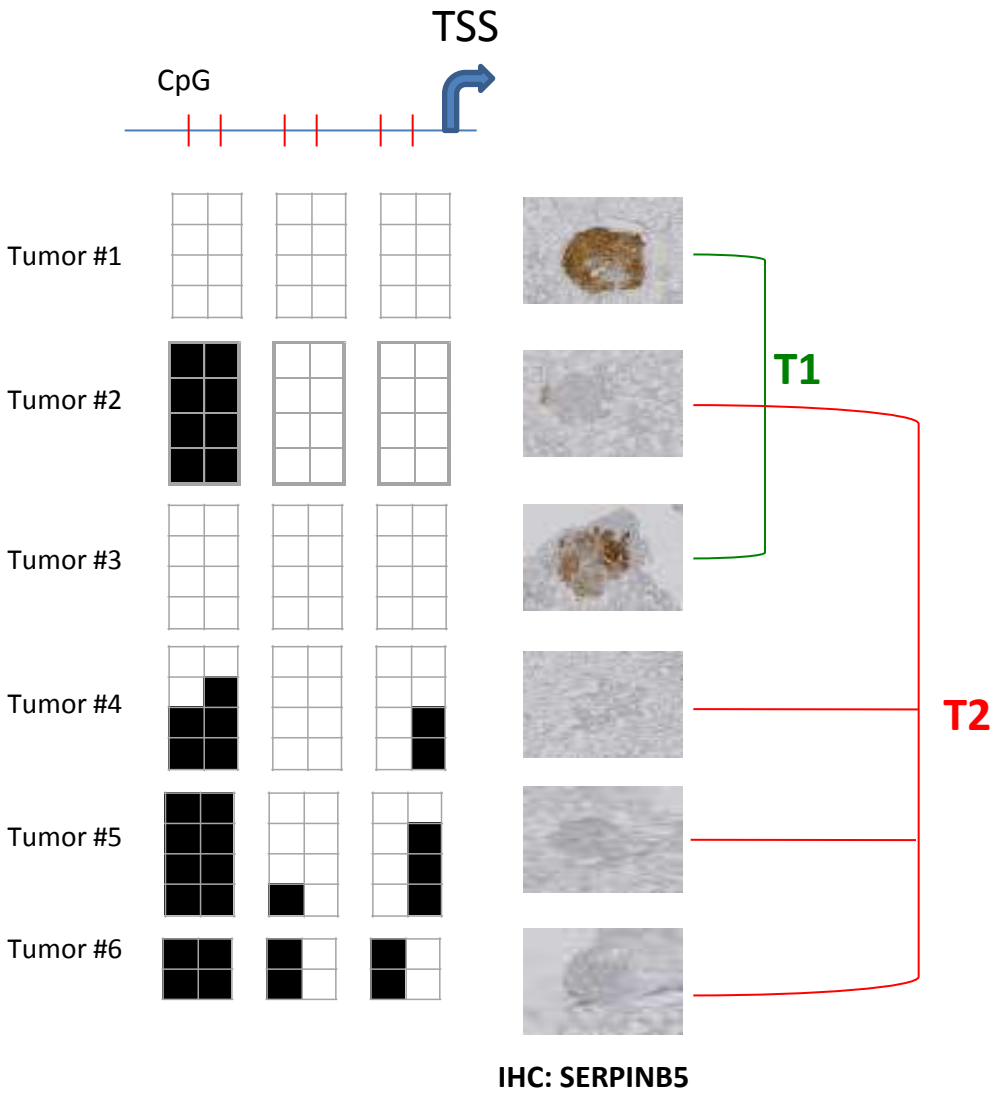
LETTERS

## An elaborate pathway required for Ras-mediated epigenetic silencing

Claude Gazin[1]*, Narendra Wajapeyee[1]*, Stephane Gobeil[1], Ching-Man Virbasius[1] & Michael R. Green[1]



# *Combination therapy: validating novel targets (II)*



# *Combination therapy: validating novel targets (II)*

5-Aza
Entinostat : reversible