

Frederick National Laboratory for Cancer Research



Ras Initiative

Frank McCormick





Structural Biology and Biochemistry

The structural and biochemical properties of KRAS and its most prevalent mutants will be characterized to look for ways to modulate their activity.

RAS Assays

New assays for RAS activity may be useful tools to screen for RAS pathway inhibitors.

Biology of Mutant KRAS Cell Lines

Commonalities in dozens of cell lines derived from human cancers that have mutant *KRAS* genes could reveal insights into selective vulnerabilities for treatment.

Pathways Analysis

Surprising failures of new cancer treatments have made it clear that we do not know enough about how molecules in RAS signaling pathways interact with each other.

Cell Surface Analysis

Identifying cell surface features specific to mutant *KRAS* cancers could give us unique opportunities to develop treatments that target the cell surface.

RAS Reference Reagents

An important priority of the RAS Initiative is to distribute highly validated materials and methods to the world-wide community of RAS researchers.

MORE THAN

30%

OF ALL HUMAN CANCERS
ARE DRIVEN BY MUTATIONS OF

RAS GENES

RAS MUTATIONS

IN HUMAN CANCERS

	PANCREAS – KRAS	95%
	COLORECTAL – KRAS	45%
	LUNG – KRAS	35%
	AML – NRAS	30%
	MELANOMA – KRAS	15%
	BLADDER CANCER – NRAS	15%

“RAS ONCOGENES ARE
THE **WORST** ONCOGENES.”

— Dr. Frank McCormick,
RAS National Program Advisor

Implementing the RAS Program

Hub, Spoke, and RAS Community model

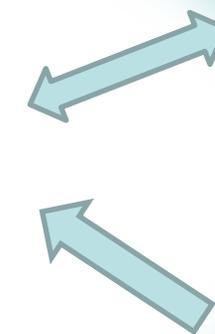
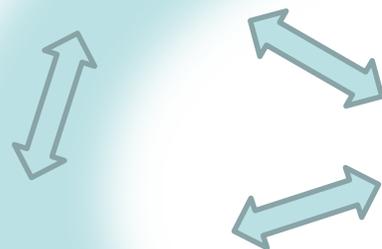
Intramural Labs



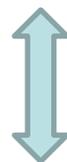
Extramural NCI-Supported Labs



FNLCR – The Hub



Biotechs



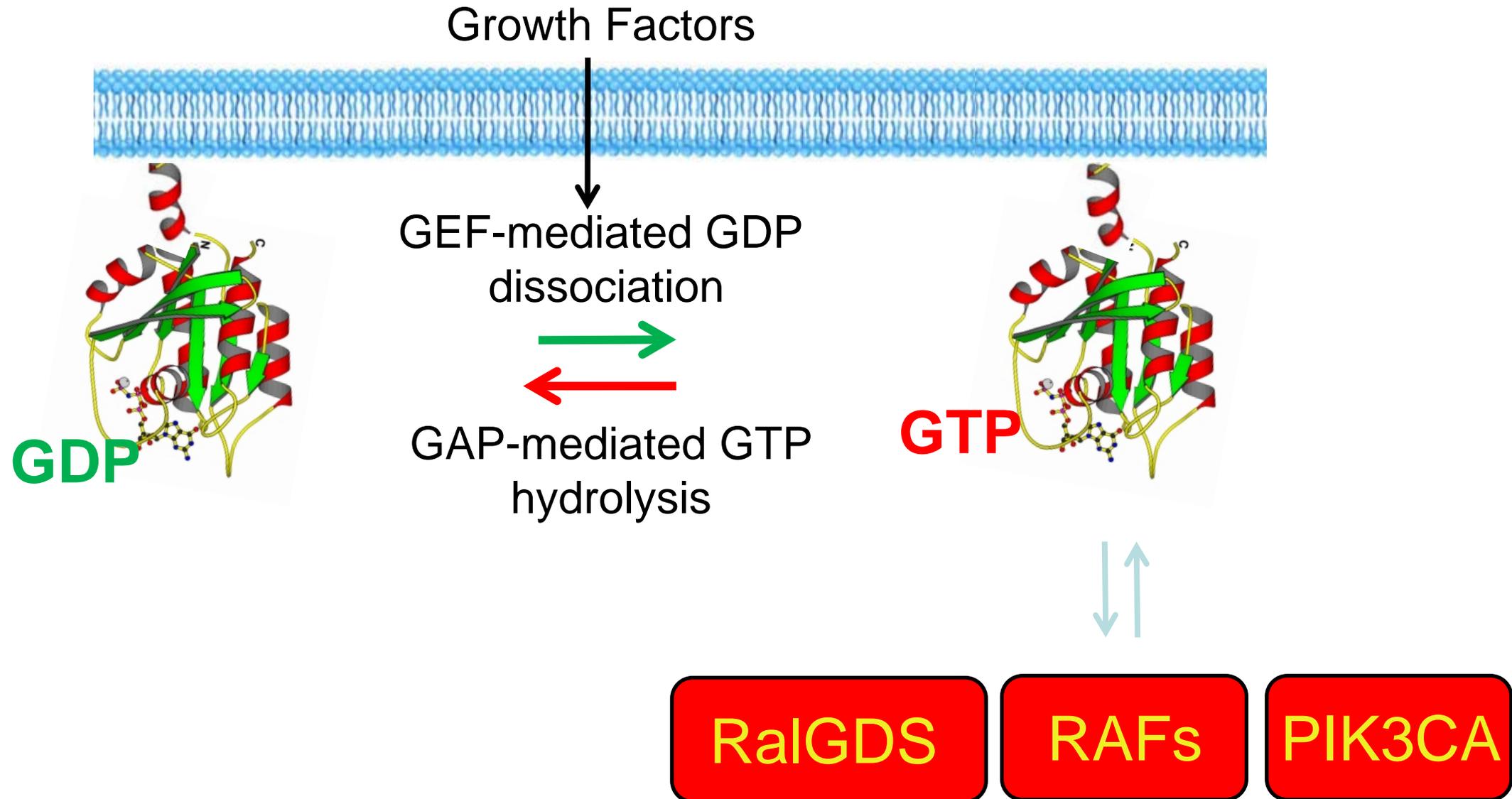
Pharma

**PANCREATIC CANCER
ACTION NETWORK™**

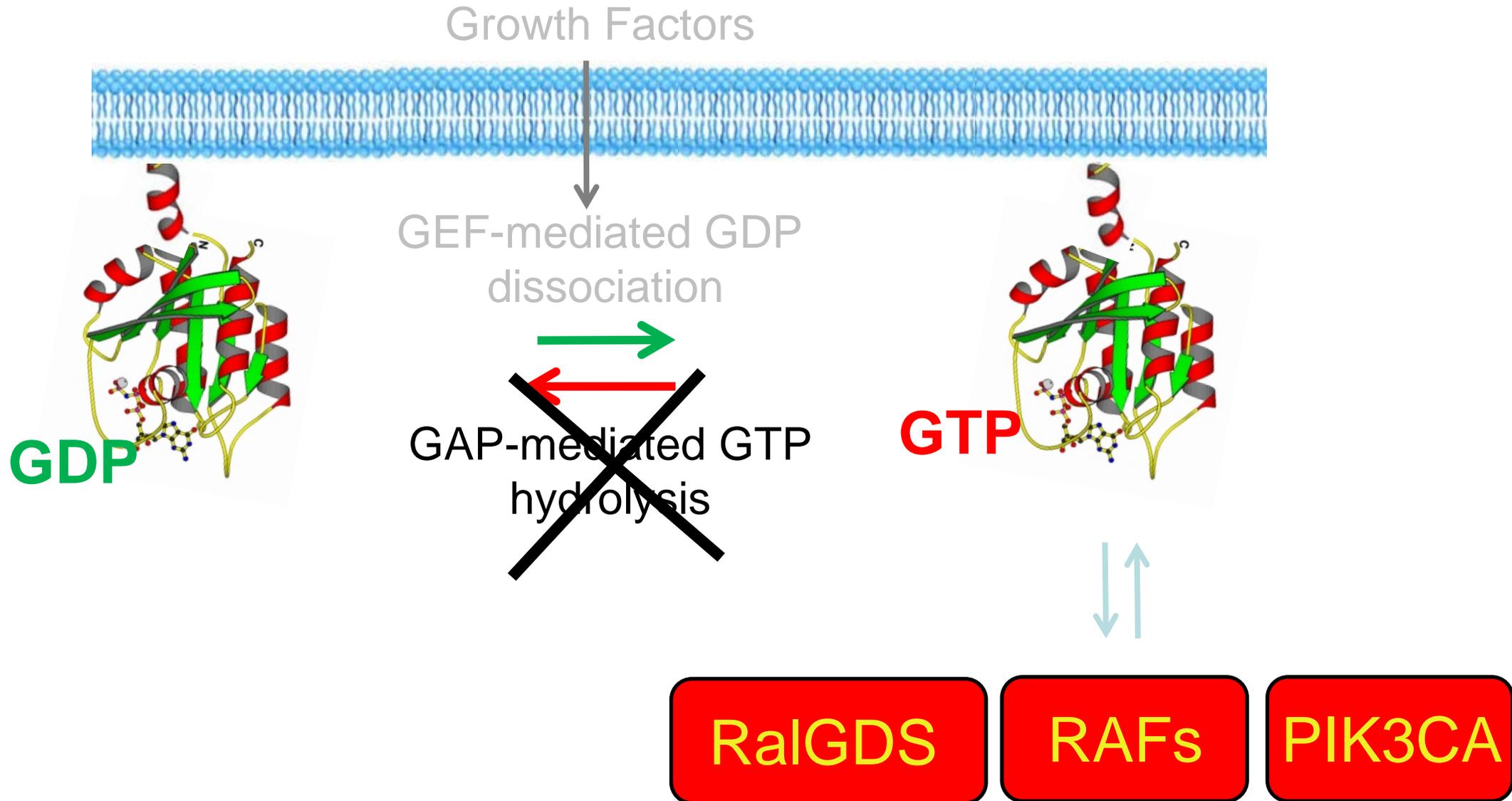


Advocacy

Parameters affecting normal Ras activity

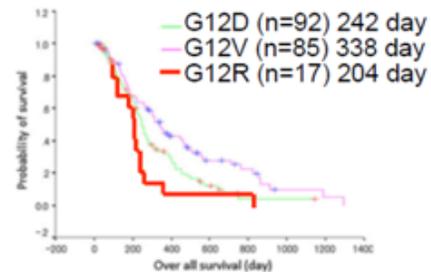


Parameters affecting oncogenic Ras activity



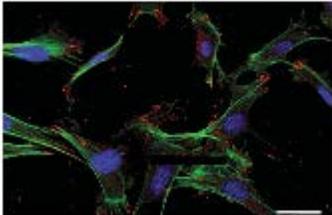
Distinct biological and clinical properties of KRAS alleles

- KRAS G12V, G12C: worse clinical outcome than G12D (lung cancer)
(Al-Mulla et al; Andreyev et al; Vega et al; Keohavong et al)
- KRAS G13D: respond to Cetuximab (colorectal cancer)
(de Roock et al, 2010)



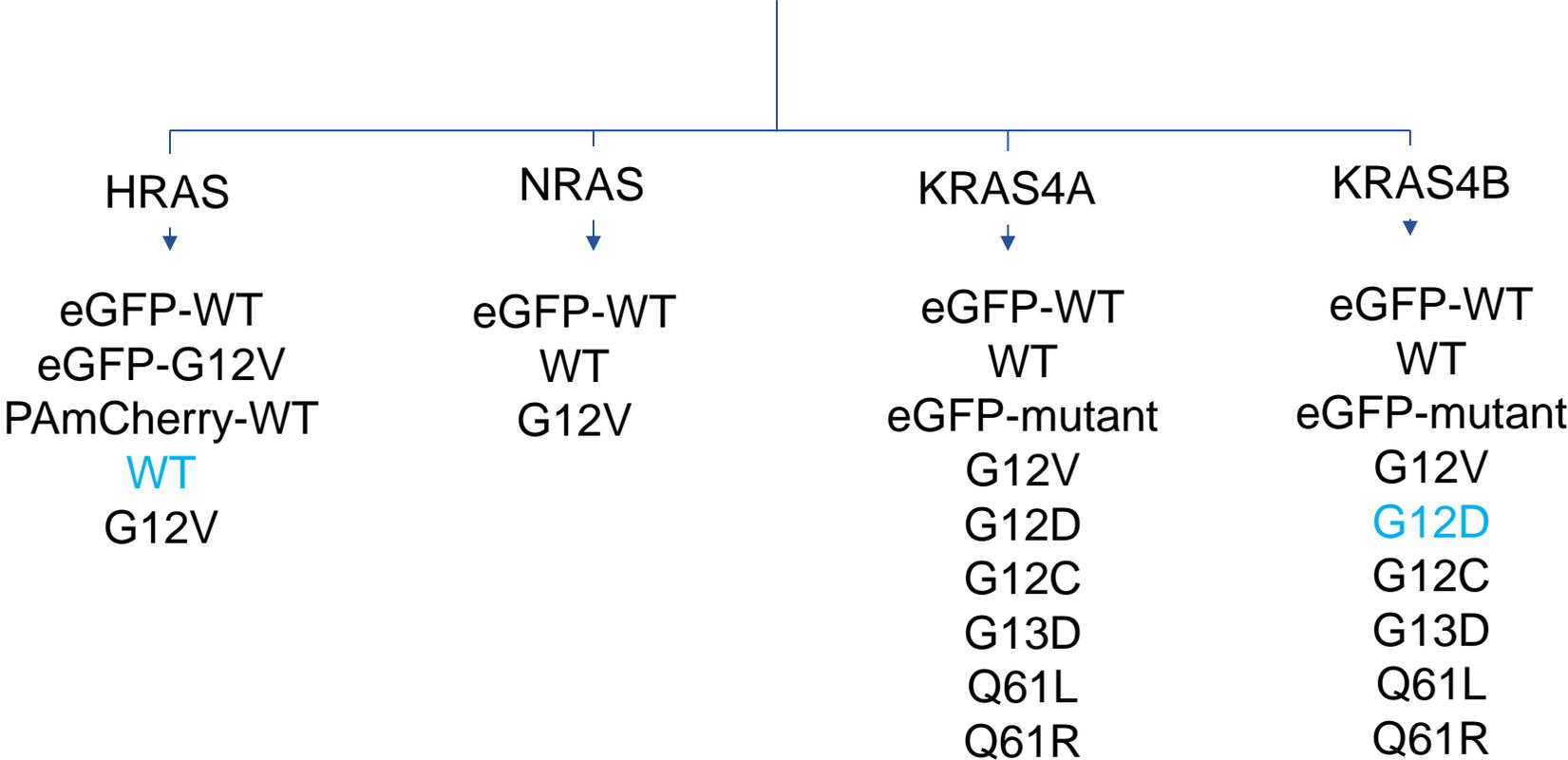
T Ogura *et al.* *J Gastroenterol* (2013)
doi:10.1007/s00535-012-0664-2

Isogenic cell lines from RAS-less MEFs



HRAS^{-/-} NRAS^{-/-} KRAS^{lox/lox} MEFs

Drostén M, Dhawahir A, Sum EY, Urosevic J,
Lechuga CG, Esteban LM, Castellano E,
Guerra C, Santos E, **Barbacid M.**
EMBO J. 2010

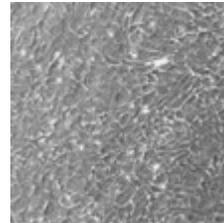


RAS dependent MEFs

HRAS^{-/-} NRAS^{-/-} KRAS^{lox/lox} MEFs

Drosten M, Dhawahir A, Sum EY, Urosevic J,
Lechuga CG, Esteban LM, Castellano E,
Guerra C, Santos E, **Barbacid M.**
EMBO J. 2010

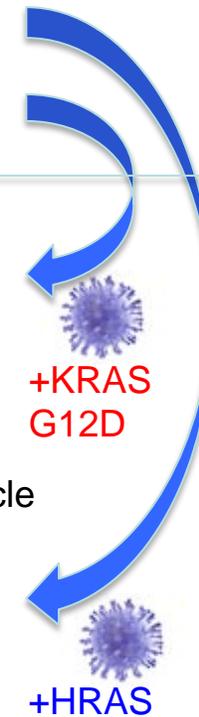
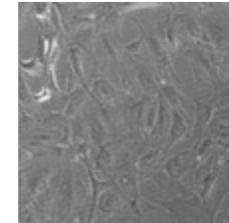
Untreated MEFs



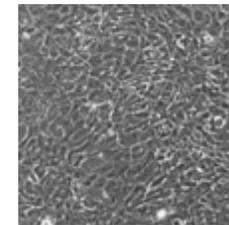
+4-OHT



G1 arrest (day 19*)



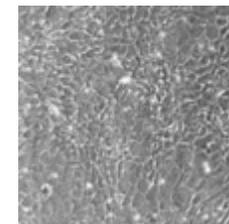
+ drugs



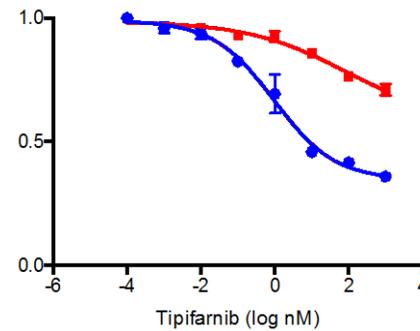
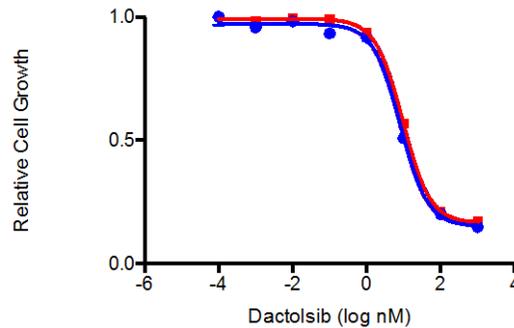
+KRAS
G12D

Re-enter cell cycle

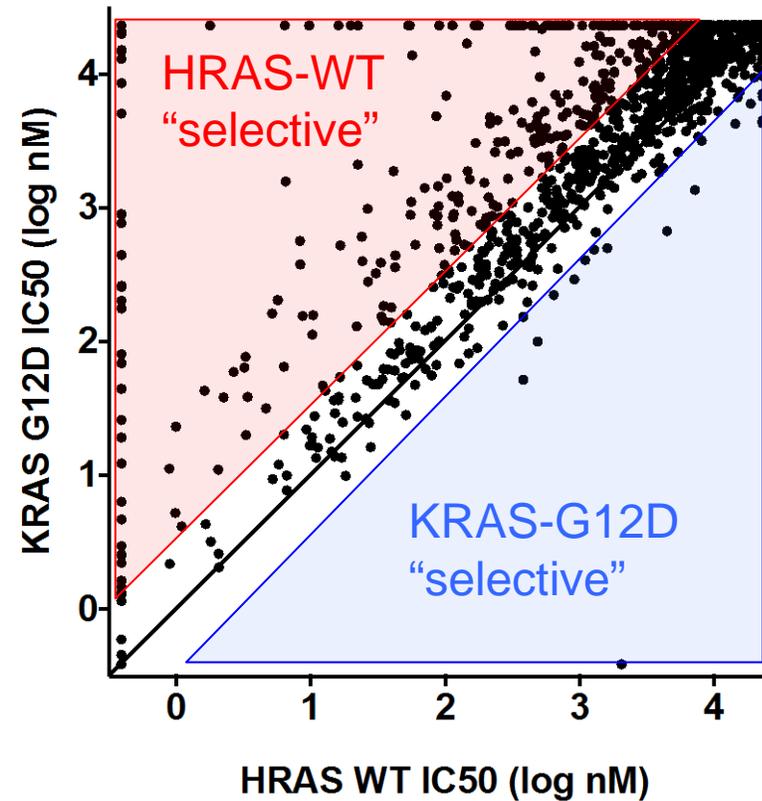
+ drugs



+HRAS

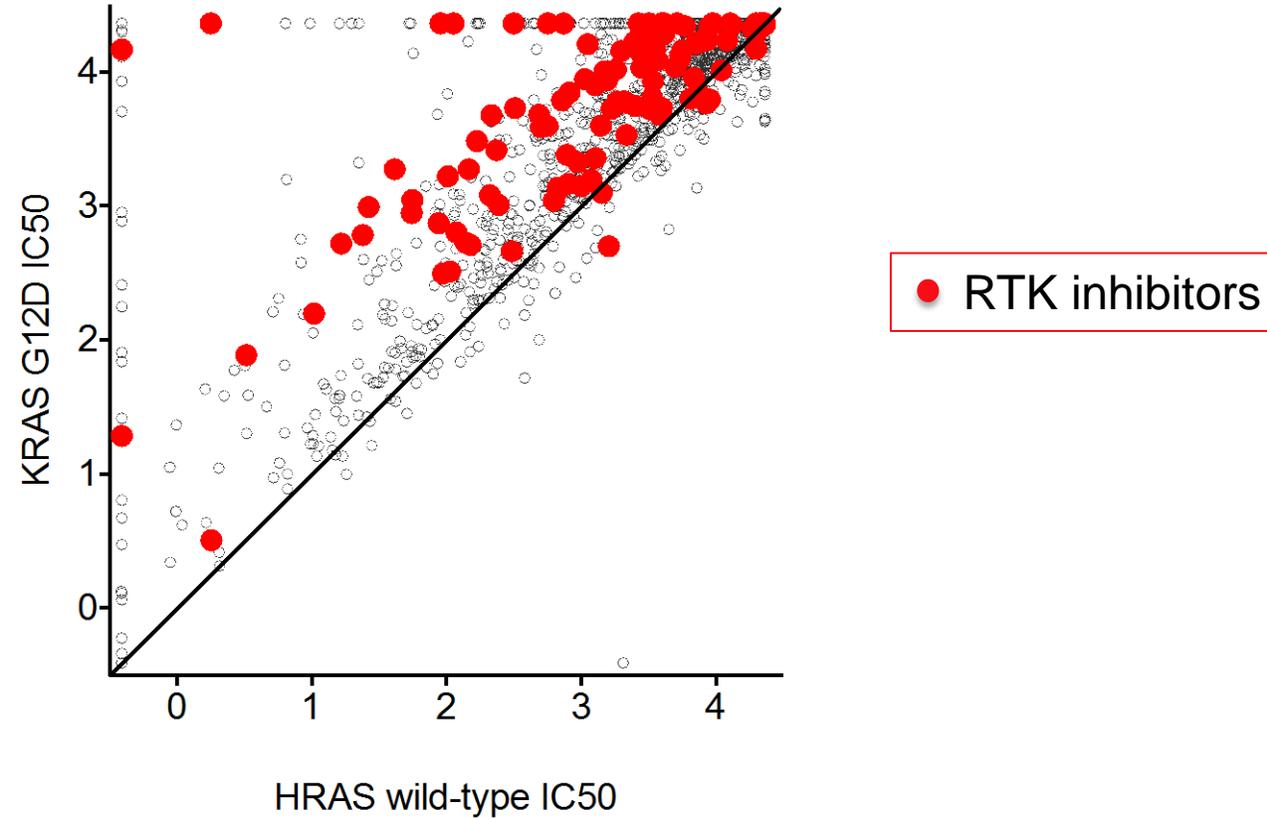


HRAS^{WT} vs KRAS^{G12D} MEF proliferation screen



- Compound library was provided by NCATS (National Center for the Advancement of Translational Sciences)
- The library is enriched for "tool" compounds, but also contains FDA approved drugs

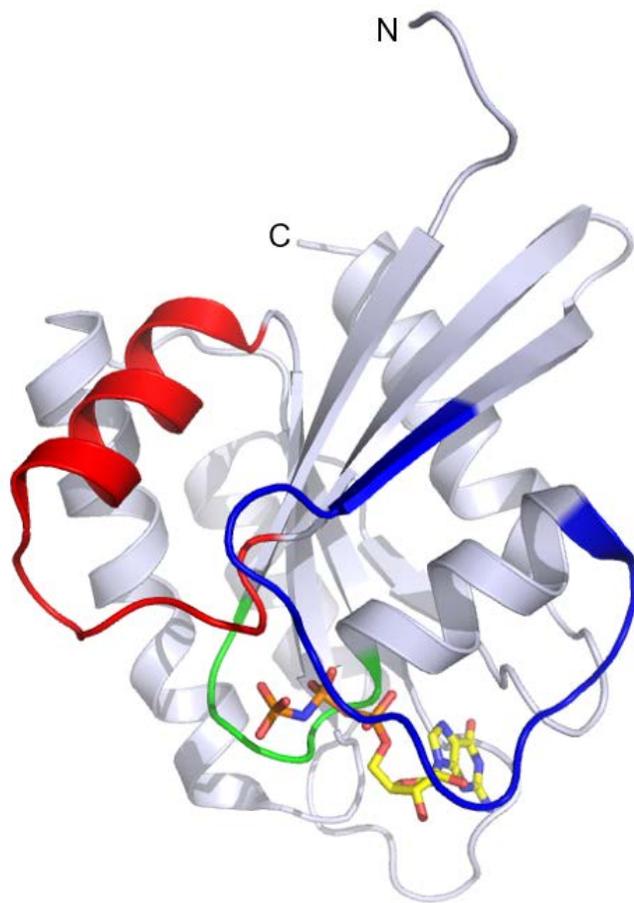
Receptor Tyrosine Kinase (RTK) inhibitors



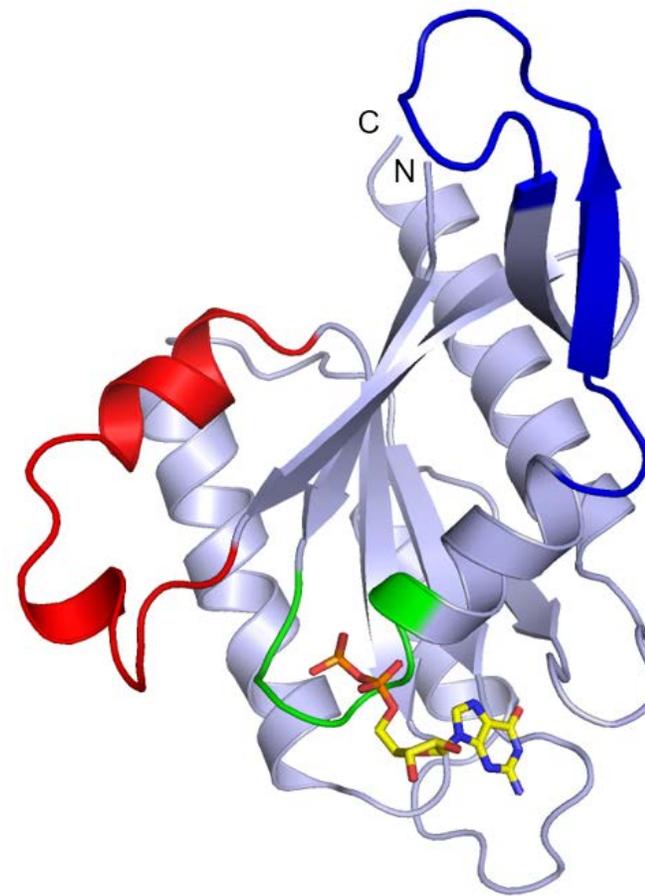


Full-length KRAS in complex with GDP

Wild-type KRAS(1-166)-GMPPNP complex at 1.35 Ang



Full-length Wild-type KRAS-GDP complex at 1.6 Ang

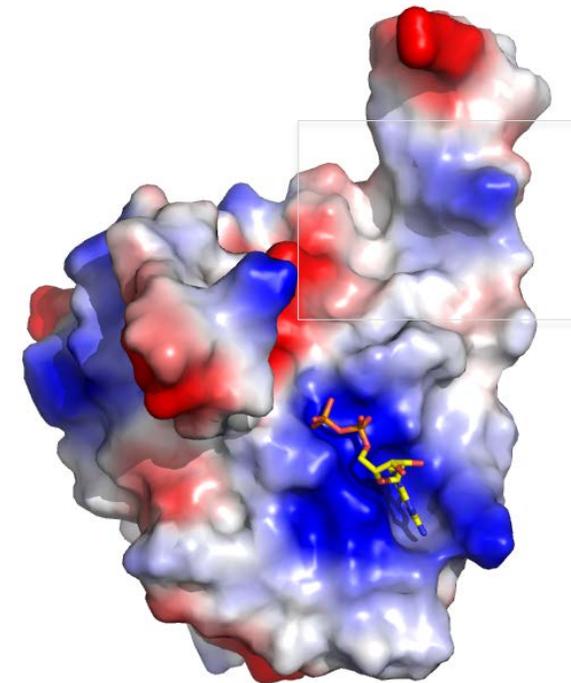


Switch-I
Switch-II
P-loop

Extended switch-I conformation in KRAS

- **Validate presence of extended switch-I conformation in solution by NMR.**
 - Dynamic studies in collaboration with National Magnetic Resonance Facility at Madison.
 - *Que Van at FNLCR*
 - High-pressure NMR studies in collaboration with Dr. Kalbitzer, University of Regensburg, Germany.
- **Virtual compound screening to target the groove present at the base of switch-I region**
 - in collaboration with Dr. Brian Shoichet's group at UCSF.

Electrostatic surface

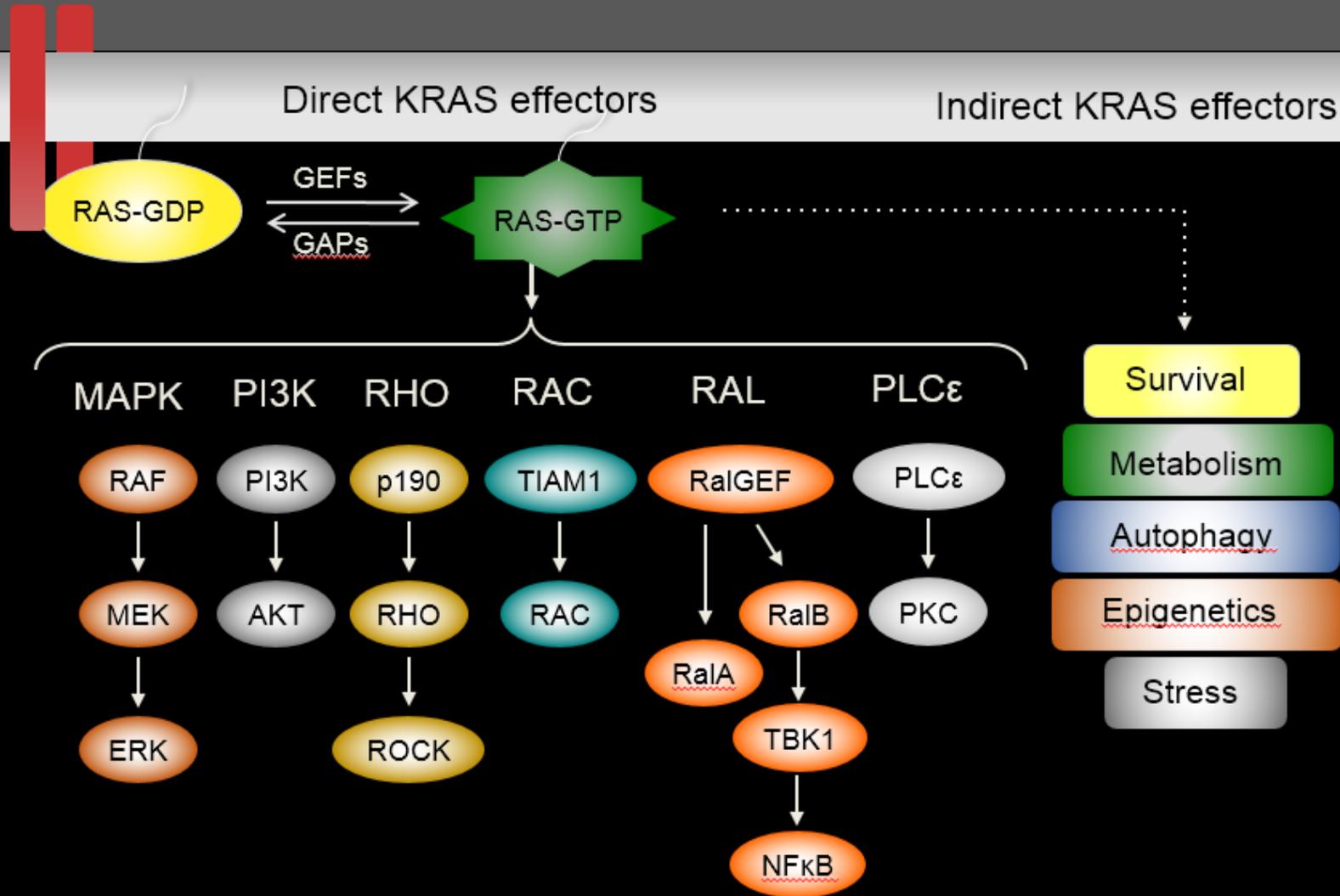


Red - negative charge

White - neutral

Blue - positive charge

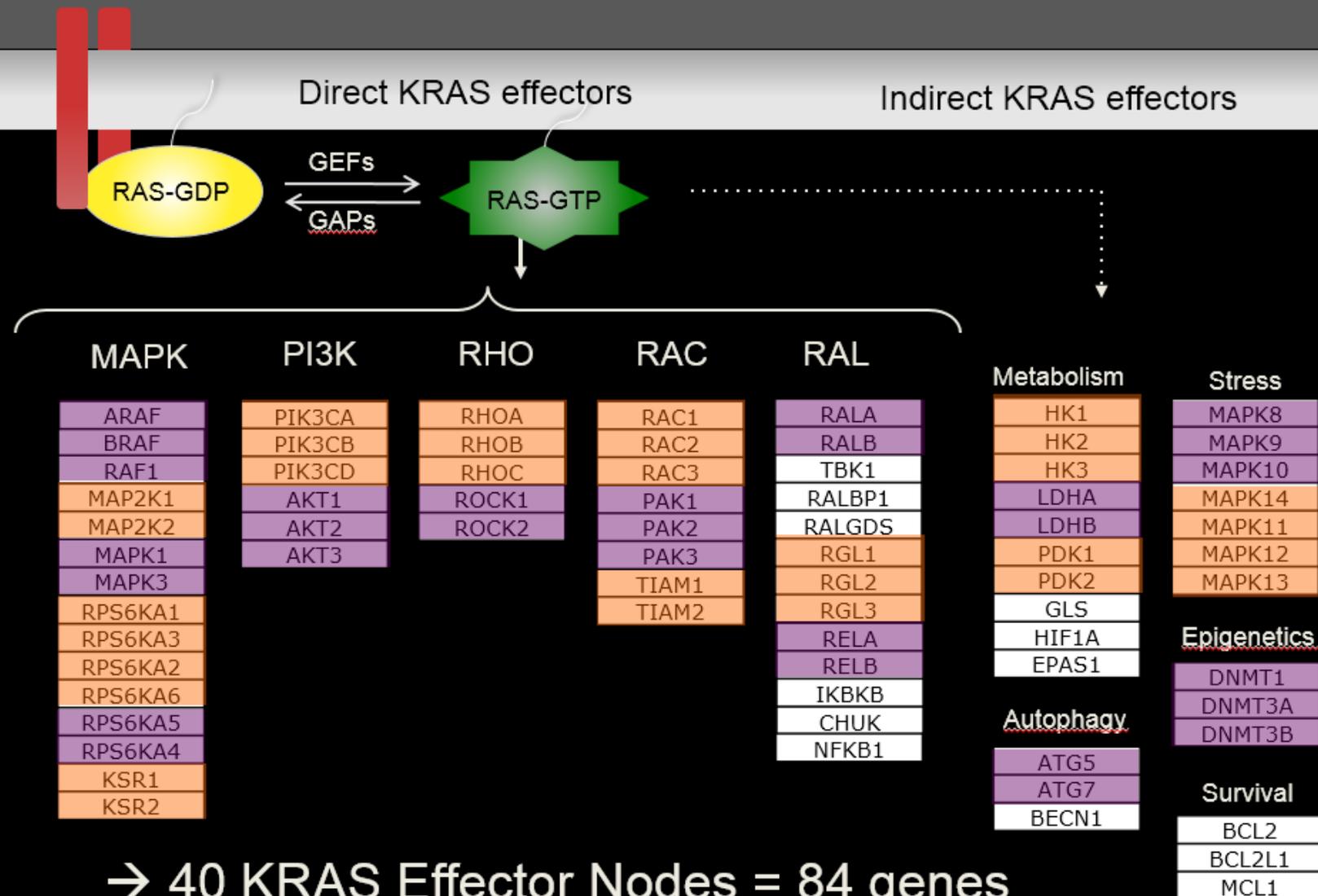
KRAS Effector Signaling: An extensive and complex network



→ 40 KRAS Effector Nodes

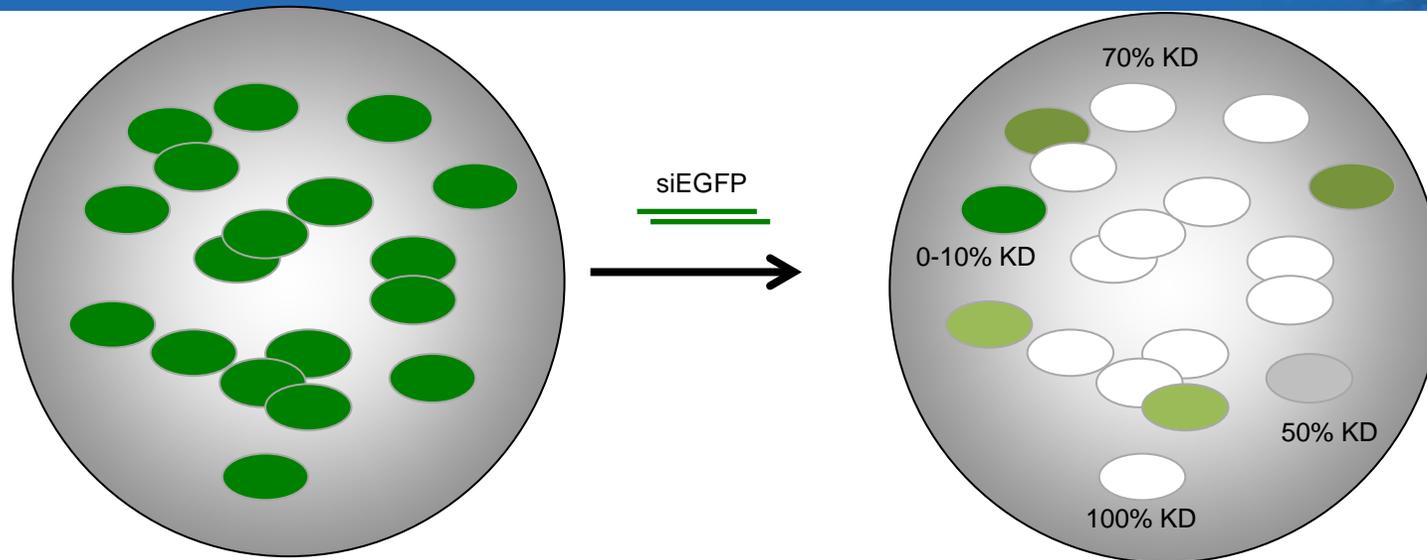


✧ Complete NODE knockdown: compensatory activation by redundant isoforms masks the importance of many nodes

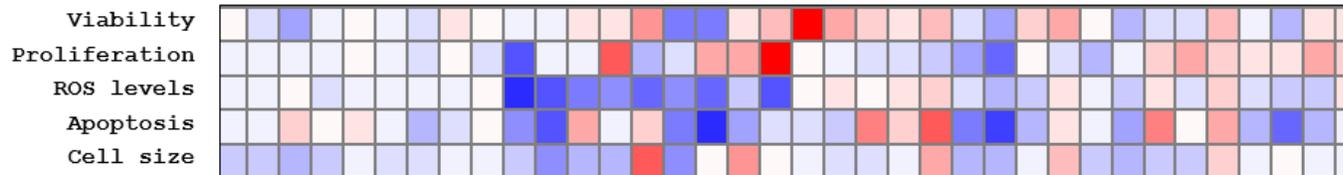


→ 40 KRAS Effector Nodes = 84 genes

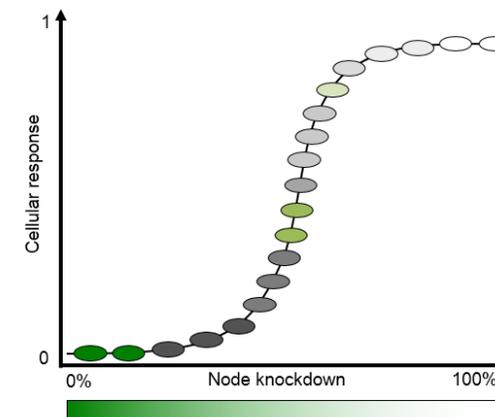
Christof Fellmann, Scott Lowe, Chih-Shia Lee, Ji Luo

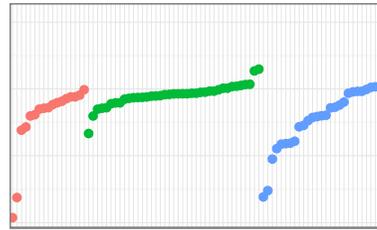


Effector Dependency Profile



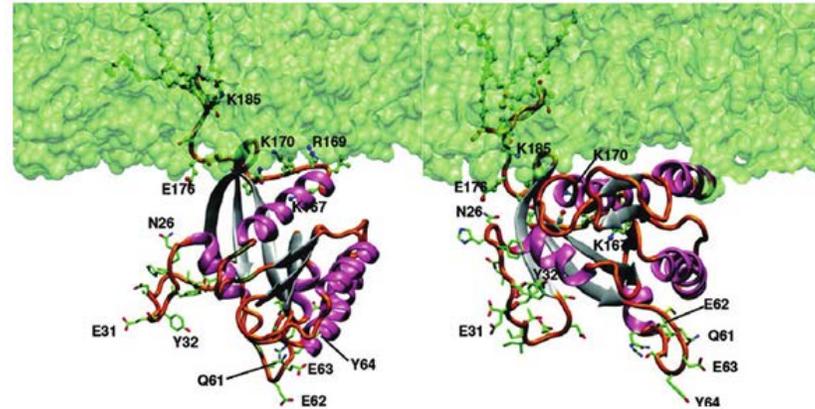
KRAS Effector NODE: 1 2 3 4 5 6 7 8 9 10 35 36 37 38 39 40





stine
Lung_NSCLC
Pancreas

Fully processed KRAS4b



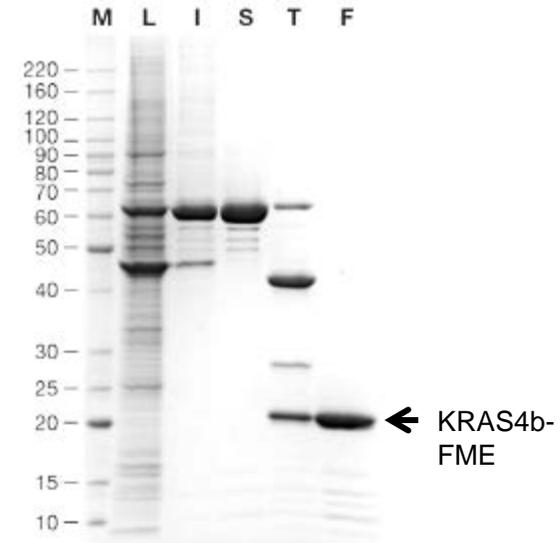
A. Gorge, U-Texas Houston

Engineering baculovirus for improved production of processed KRAS

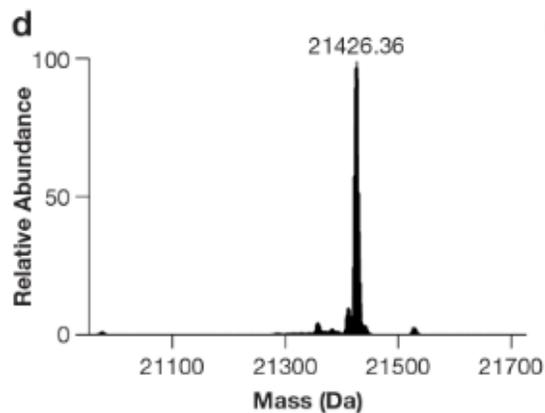
- recombineering used to insert FNTA/FNTB genes into the baculovirus genome
- eliminated issues with coinfection of multiple viruses
- maltose-binding protein (MBP) fusion for greater yield and solubility
- *Trichoplusia ni* (Hi5) insect cells for increased yield

Processed KRAS4b characterization

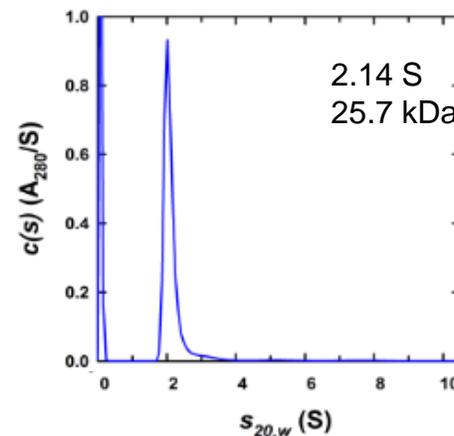
- **Extensive protein characterization**
 - Purified to homogeneity; yield >7mg/L
 - Intact mass
 - Predominantly monomeric
 - Secondary structure equivalent to non-processed KRAS4b
 - Lower thermal stability



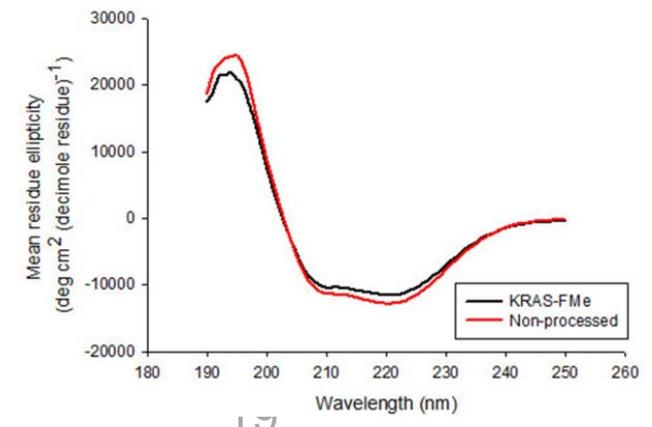
Intact mass analysis



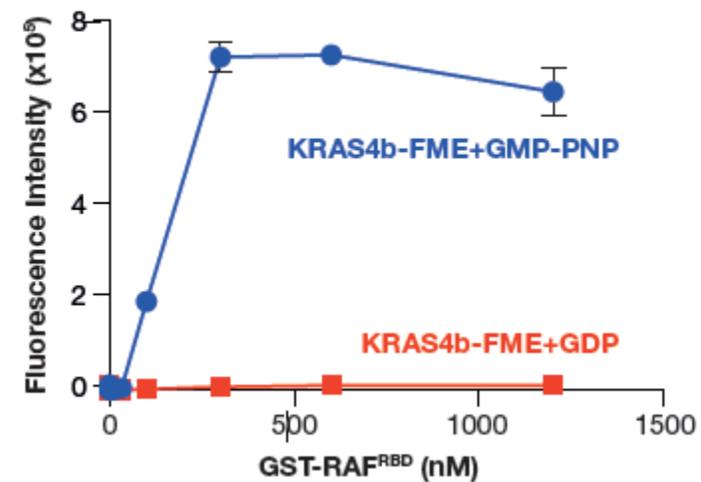
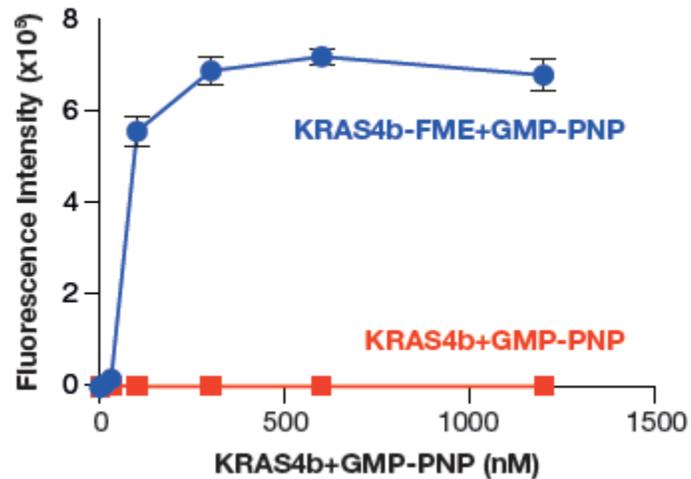
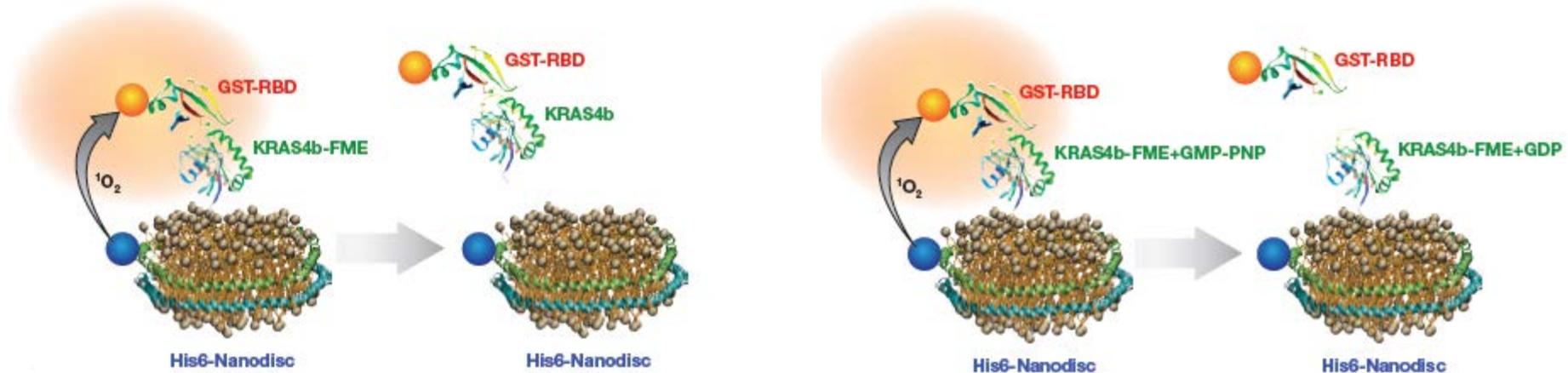
Analytical ultracentrifugation



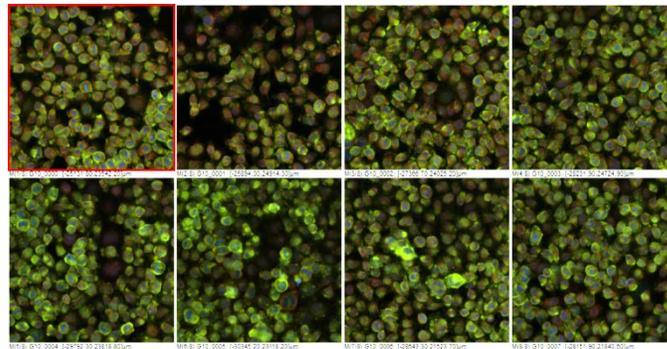
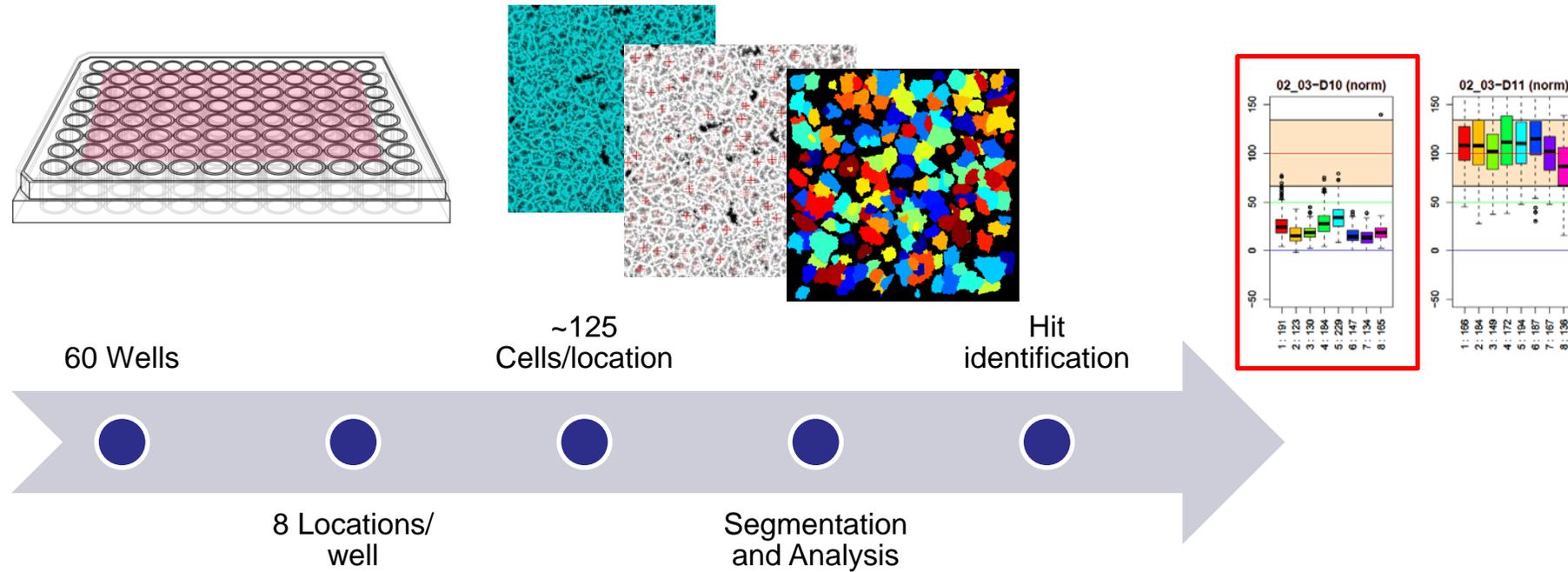
Secondary structure by CD



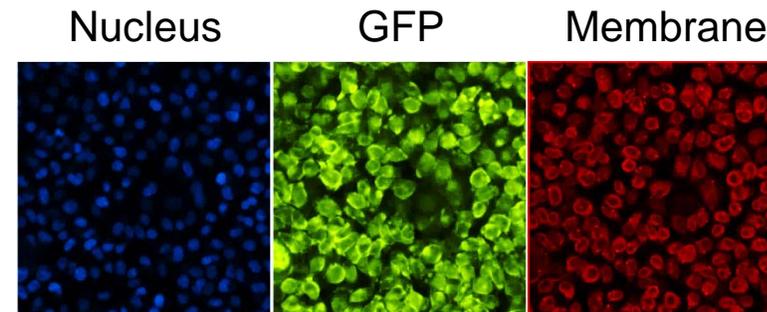
KRAS4b-FME binds to CRAF-RBD on Nanodiscs



RAS Localization Assay Overview



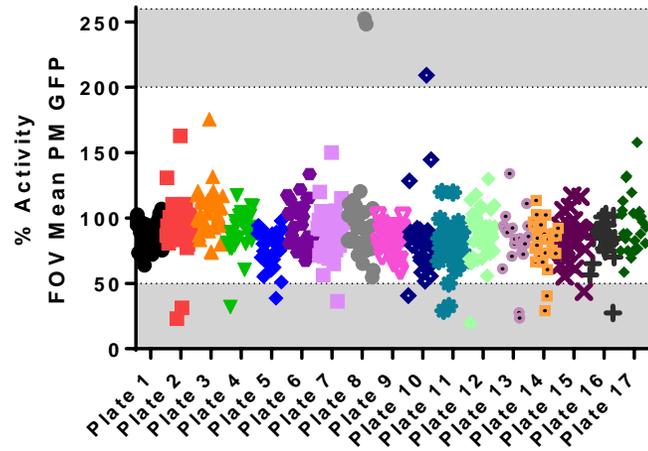
GFP-KRAS4b^{G12V}



Alla Brafman

NCI Developmental Therapeutics Program screening set

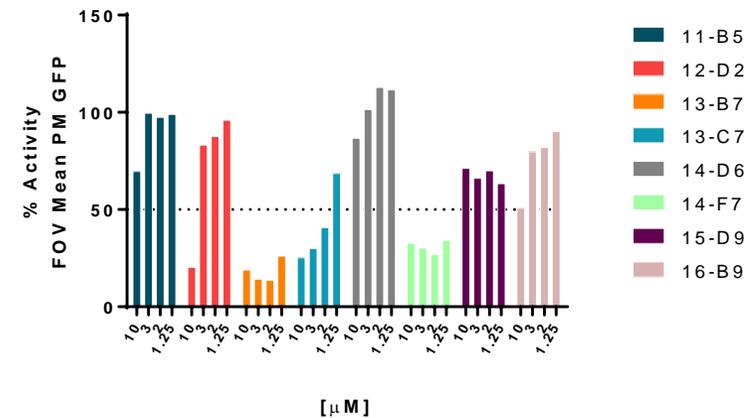
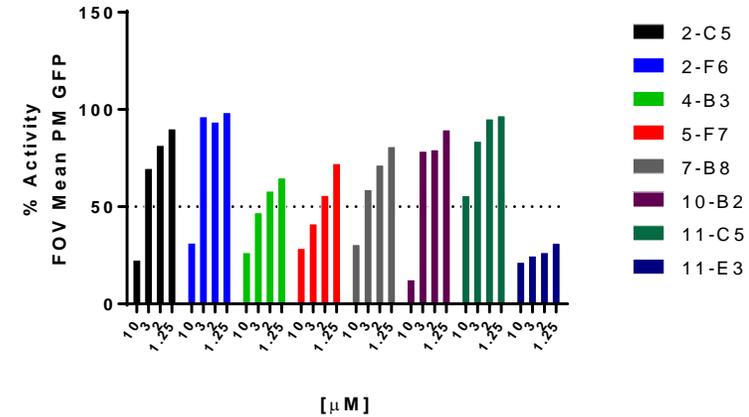
Primary assay: GFP-KRAS^{G12V}



~800 small molecules with
biological activity

- Plate 1 (Z' = 0.84)
- Plate 2 (Z' = 0.77)
- ▲ Plate 3 (Z' = 0.89)
- ▼ Plate 4 (Z' = 0.80)
- ◆ Plate 5 (Z' = 0.79)
- Plate 6 (Z' = 0.68)
- Plate 7 (Z' = 0.64)
- Plate 8 (Z' = 0.84)
- ▼ Plate 9 (Z' = 0.74)
- ◆ Plate 10 (Z' = 0.73)
- Plate 11 (Z' = 0.83)
- ◆ Plate 12 (Z' = 0.74)
- Plate 13 (Z' = 0.83)
- Plate 14 (Z' = 0.79)
- ✕ Plate 15 (Z' = 0.88)
- Plate 16 (Z' = 0.74)
- ◆ Plate 17 (Z' = 0.88)

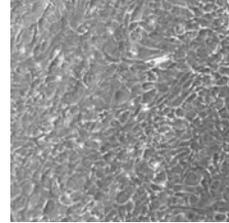
Reconfirmed hits



HRAS^{-/-} NRAS^{-/-} KRAS^{lox/lox} MEFs

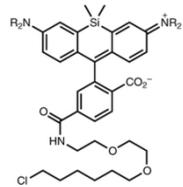
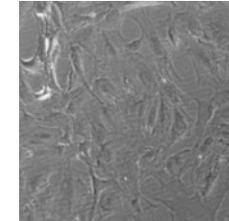
Drosten M, Dhawahir A, Sum EY, Urosevic J,
Lechuga CG, Esteban LM, Castellano E,
Guerra C, Santos E, **Barbacid M.**
EMBO J. 2010

Untreated MEFs

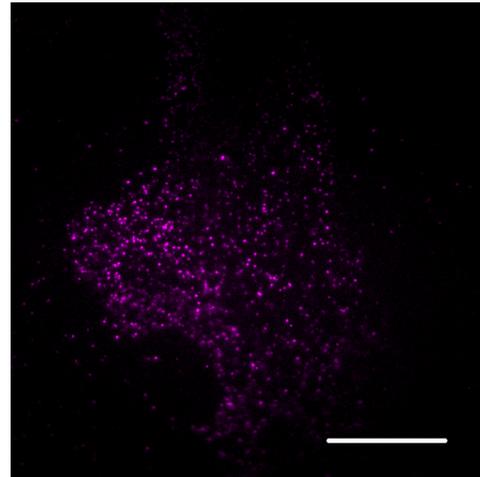


+4-OHT
→

G1 arrest (day 19*)

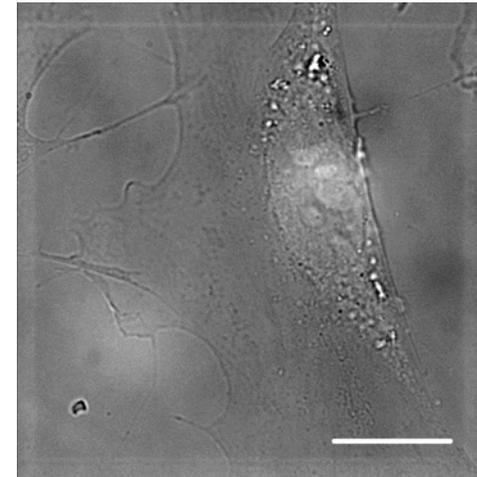


HaloTag-KRAS4b can be
imaged in live cells.



TIRF Image: membrane

HaloTag-KRAS4b rescues
RASless MEF proliferation.



Transmitted light image



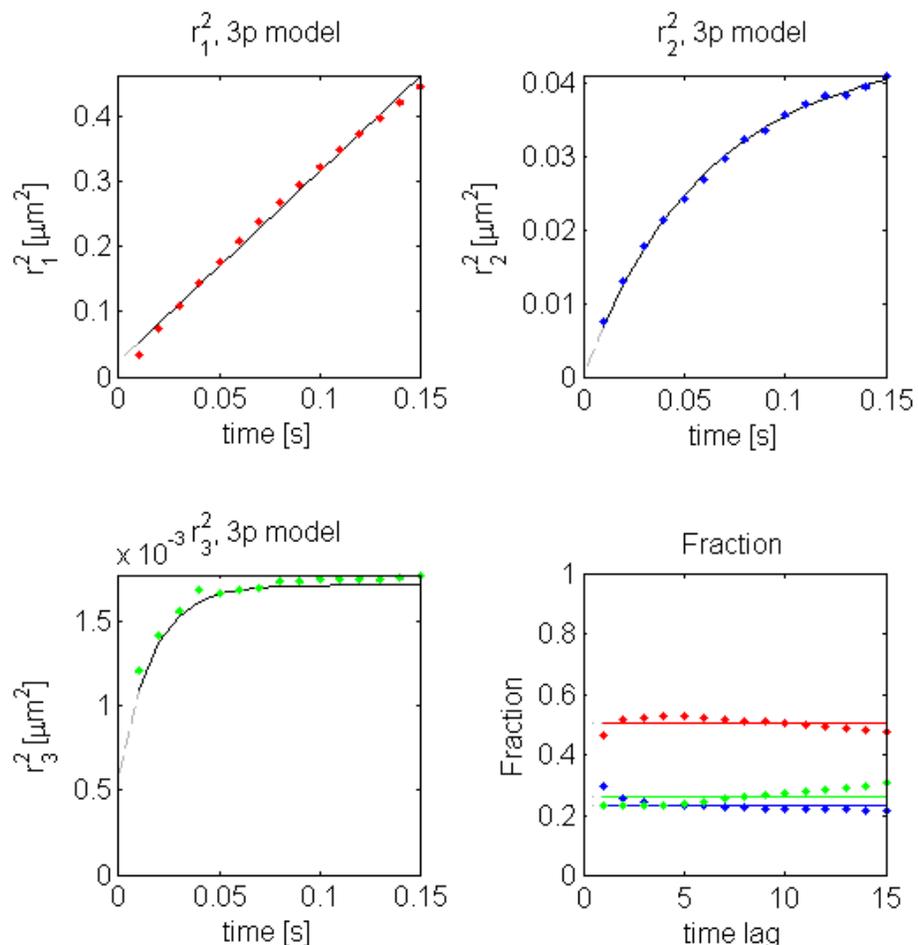
+HaloTagKRAS

Scale bar 20 μm

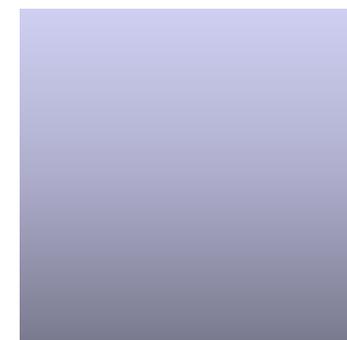
Cell permeant,
super bright,
fluorescent Halo
ligand from
Janelia Farms

Characterization of RAS molecules in live cell membranes

Jump squared displacement analysis



HaloTag-KRAS^{WT} driven-MEFs

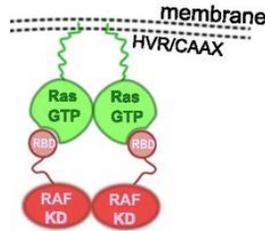


Three components

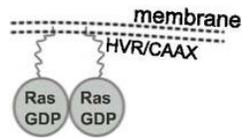
Model	Diffusion ($\mu\text{m}^2/\text{s}$)	Fraction Mean (SDev)	Const. Rad. R_c (nm)
1 \rightarrow Normal	0.73	0.505 (0.0193)	-
2 \rightarrow Constrained	0.1805	0.233 (0.021)	44.2
3 \rightarrow Constrained	0.0178	0.2624 (0.026)	1.2

Single molecule tracking analysis suggests three RAS states in live cell membranes.

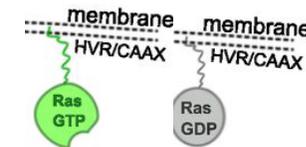
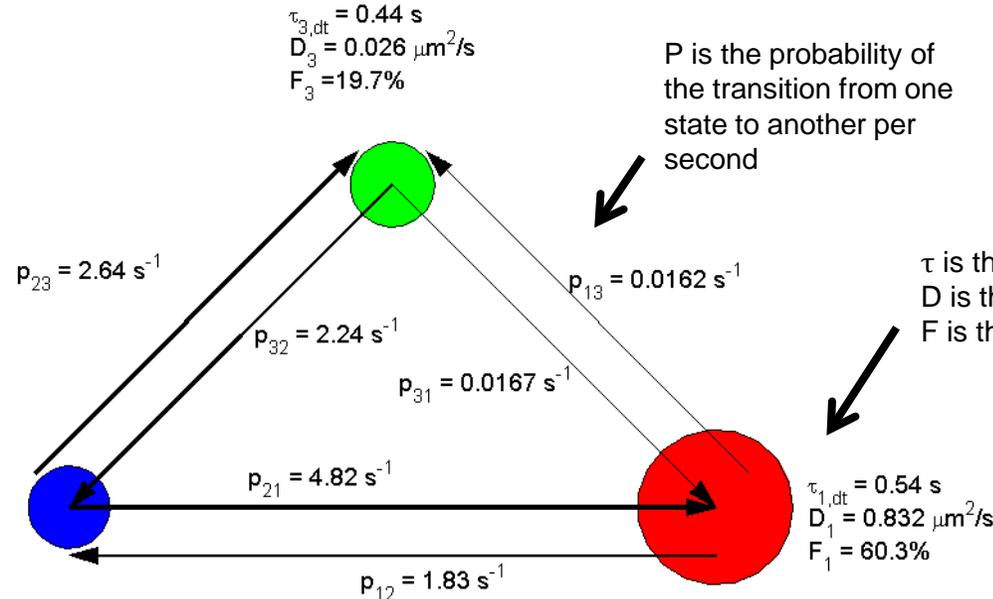
Information extracted from individual trajectories



Hypothesis: states represent different complexes in membrane.



$\tau_{2,dt} = 0.13$ s
 $D_2 = 0.183 \mu\text{m}^2/\text{s}$
 $F_2 = 20.0 \%$



RASless-MEFs, HaloTag-wtKRAS4b [JF646]=50pM, Serum Starved, 37°C, 22,325 trajectories and average trajectory length 12 frames.

Collaboration with the RAS Community

RAS events

Synthetic Lethality Workshop, January 6-7 2014
 RAS Pathways Workshop, June 11, 2014
 Cell Surfaces Workshop, July 23, 2014
 AACR Annual Meeting, April 21, 2015
 RAS Structures Workshop, July 21-22, 2015
 RAS Immunotherapy Workshop, November 3, 2015
 RAS Symposium, December 15-16, 2015

Seminars at FNLCR

Channing Der, UNC
 Ken Westover, UTSW
 Carla Mattos, Northeastern
 Mark Philips, NYU
 Vadim Gaponenko, U-Chicago
 Josh Salafsky, Biodesy, Inc.
 Calvin Kuo, Stanford
 Kris Wood, Duke
 Mariano Barbacid, CNIO, Madrid
 Cyril Benes, Mass General
 Carolyn Buser, GlaxoSmithKline
 Jay Groves, UC-Berkeley
 Stephen Sligar, UI-Champaign Urbana
 Raffit Hassan, NCI
 Renata Grifantini, Externautics Spa, Siena
 Renata Pasqualini, U-New Mexico
 Andrew Bradbury, Los Alamos
 Kent Rossman, UNC
 Shiva Malek, Genentech



Outside collaborators

Steve Almo, Einstein
 Jim Wells, USCF
 Channing Der, UNC
 Ken Westover, UTSW
 Carla Mattos, Northeastern
 Steve Sligar, U- III
 Jay Groves, Berkeley
 Hirsch Nanda, Susan Kreuger, NIST
 John Markley, NMRFAM, UW-Madison
 Paul Cohen, DARPA
 Kris Wood, Duke
 David Weber, U-Maryland
 Tina Yuan, Broad
 Cameron Pitt, UCSF
 Krishna Kota, USAMRIID
 Sotirios Koutsopoulos, MIT
 Fred Wittinghofer, Dortmund University
 Lynn McGregor, UCSF (PanCan postdoc)
 John Hunter, UTSW (PanCan postdoc)
 Saori Sato, Daiichi-Sankyo
 Walter Englaro, Sanofi-Aventis
 Kirk Staschke, Lilly
 Gad Getz, Mass Gen /Broad
 Matt Meyerson, Dana Farber
 Immuno-MRM of RAS pathway

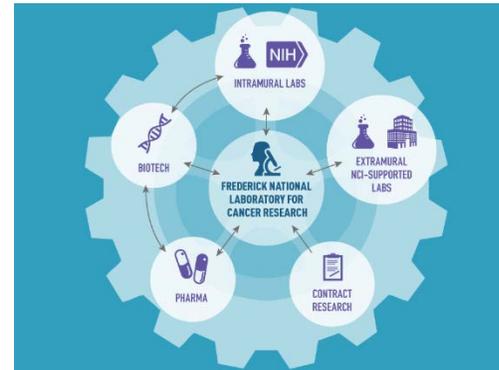
- Amanda Paulovich, Fred Hutch
- Steve Carr, Broad Institute
- John Koomen, Moffit Cancer Center

 Andreas Gosberg, Lilly

Collaboration with the RAS Community

RAS Reference Reagents

Chris Kemp, Fred Hutch
Eric Chang, Baylor
Silvia Thöne, Munich
Peter Jackson, Stanford University
Tyler Jacks, MIT
Calvin Kuo, Stanford
Bill Hahn, Broad / Dana Farber
Karla Satchell, Northwestern
Julian Downward, Cancer Research UK
Daniel Abankwas, University of Turku
Said Sebt, Moffitt Cancer Center
Ian Prior, Liverpool
Muller Fabbri, Children's Hospital LA
Faraz Bishehsari, Rush
Amy Lee, USC
Yosef Yarden, Weizmann
Richard Klemke, UCSD
Saidul Chowdhury, U-Texas Arlington
Christian Gocke, JHMI
Tobias Baumgart, U-Penn
Emil Lou, U-Minnesota
Ron Bose, Wash U
Neil Kelleher, Northwestern
Sourav Bandyopadhyay, UCSF
Robert Chapkin, Texas A&M



NIH collaborators

Ji Luo, NCI
Anton Simeonov, NCATS
Debbie Morrison, NCI
Rajat Varma, NIAID
Udo Rudloff, NCI
Sriram Subramaniam, NCI