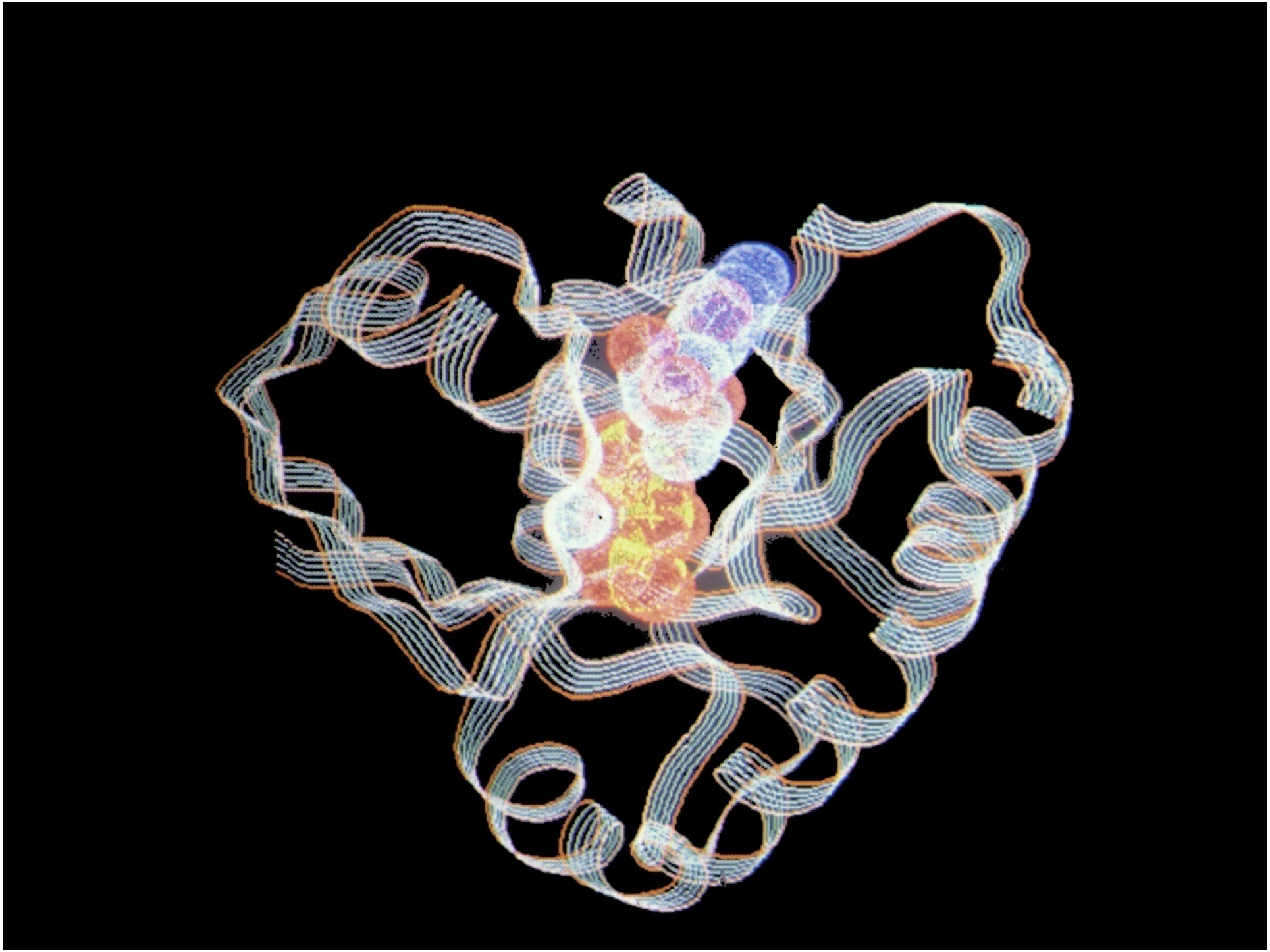
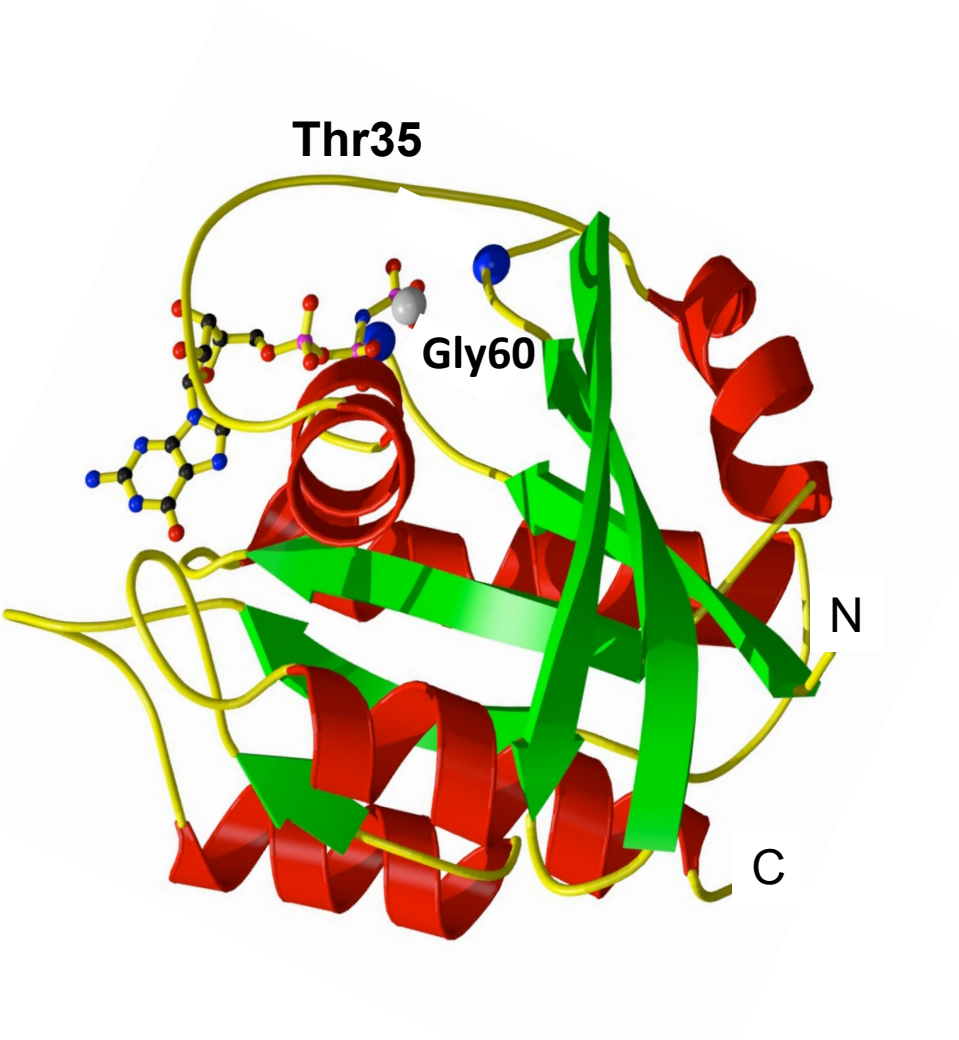
Search: Ras Central e-mail:SolveRas@nih.gov

RAS mutations in human cancer

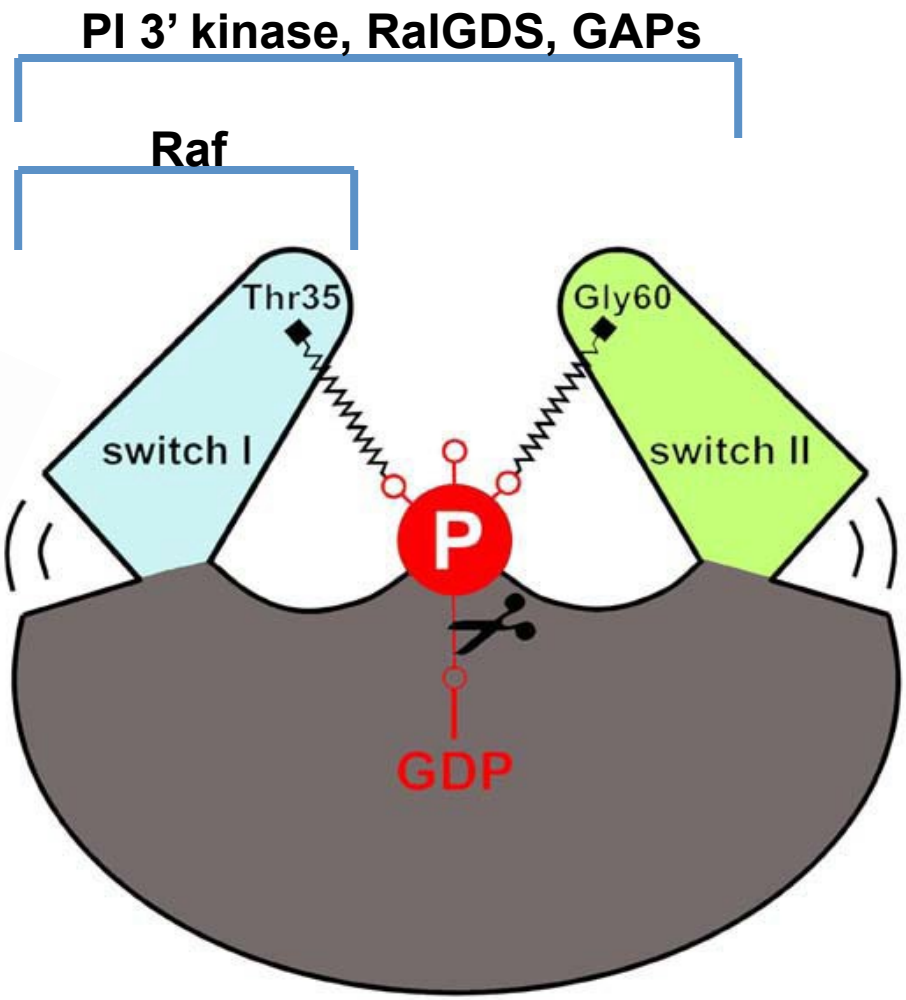
Pancreas	95%	KRAS
Colorectal	45%	KRAS
Lung	35%	KRAS
AML	30%	NRAS
Melanoma	15%	NRAS
Bladder Cancer	5%	HRAS
Thyroid Cancer	5%	HRAS



The GDP/GTP switch

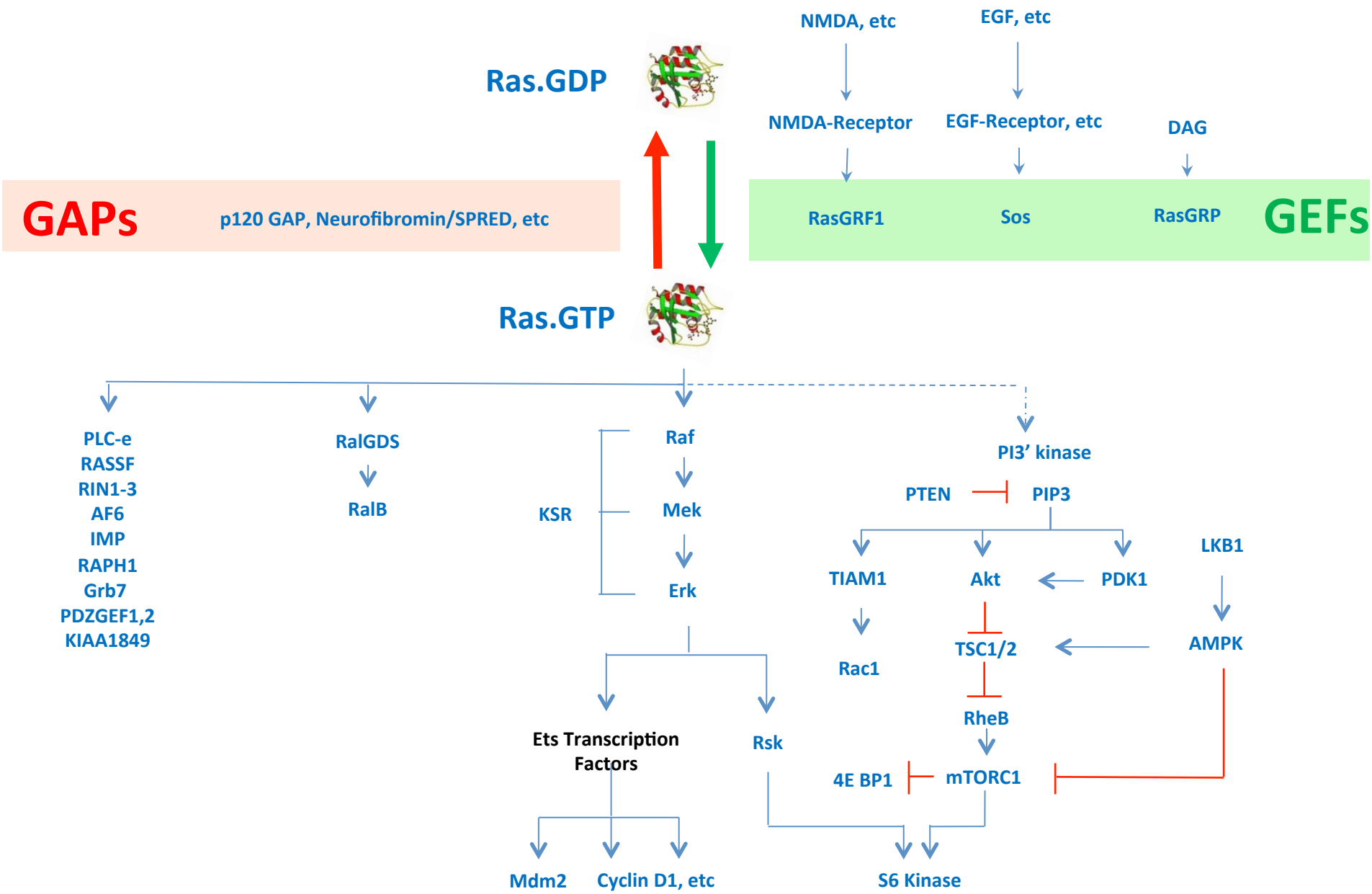


Fred Wittinghofer et al

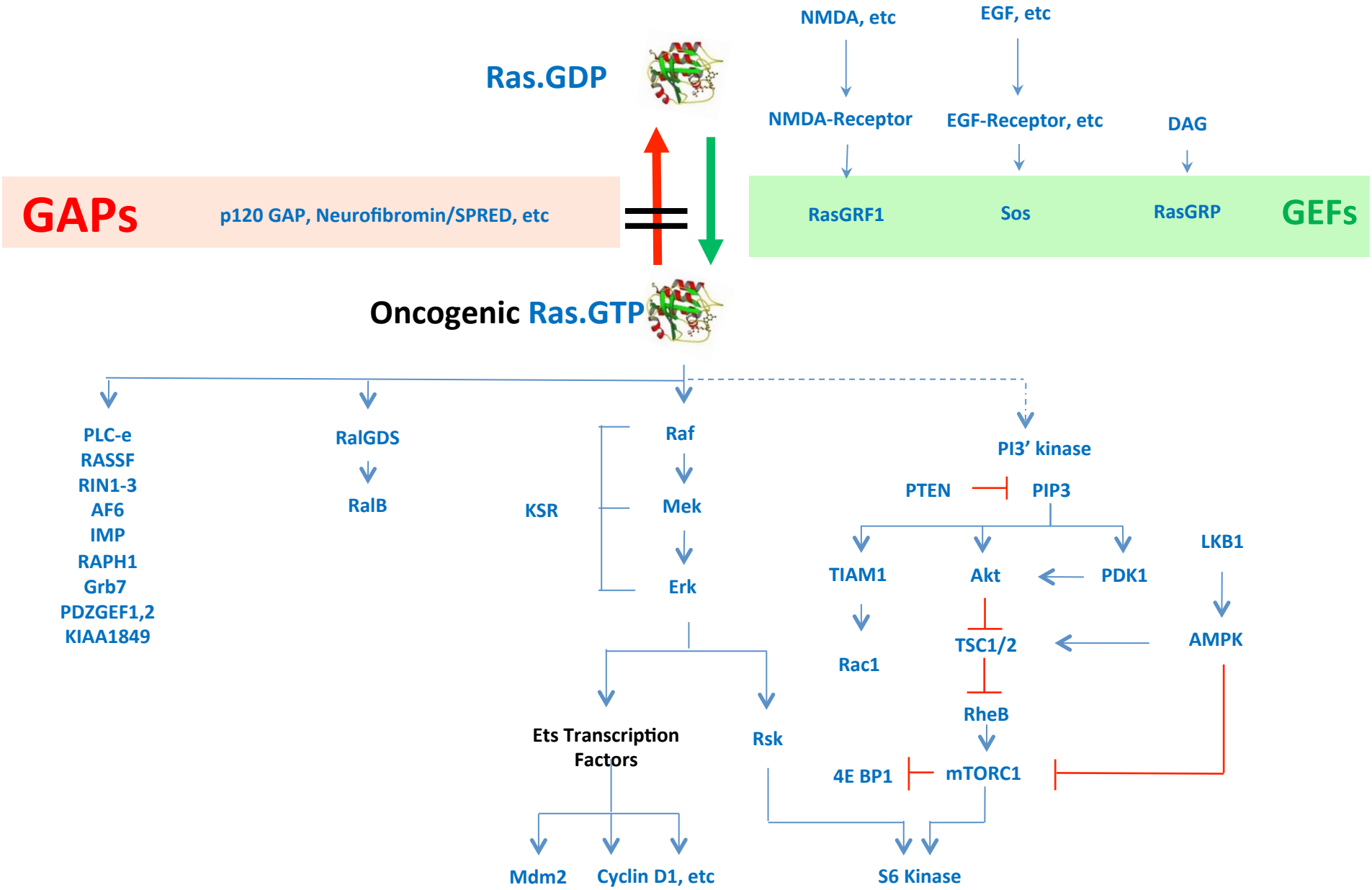


Christina Kiel

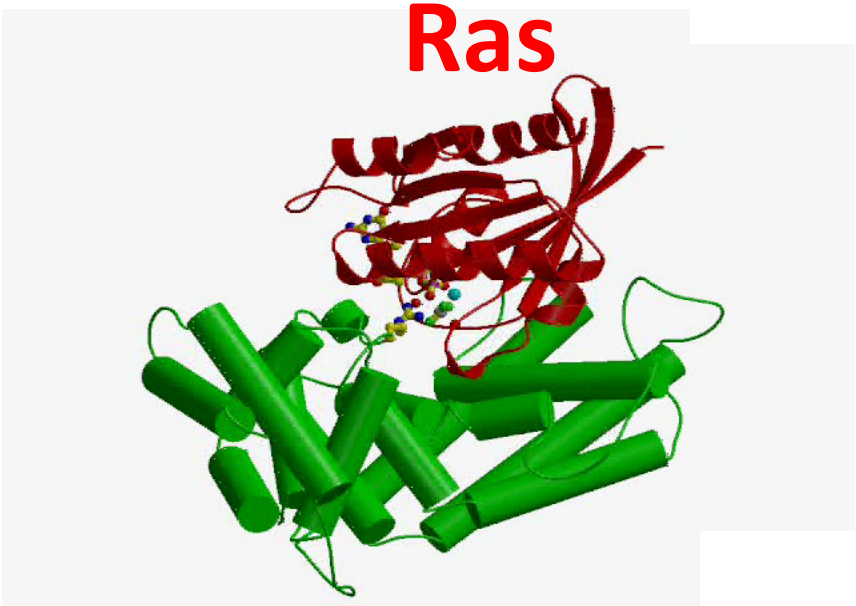
The Ras Pathway



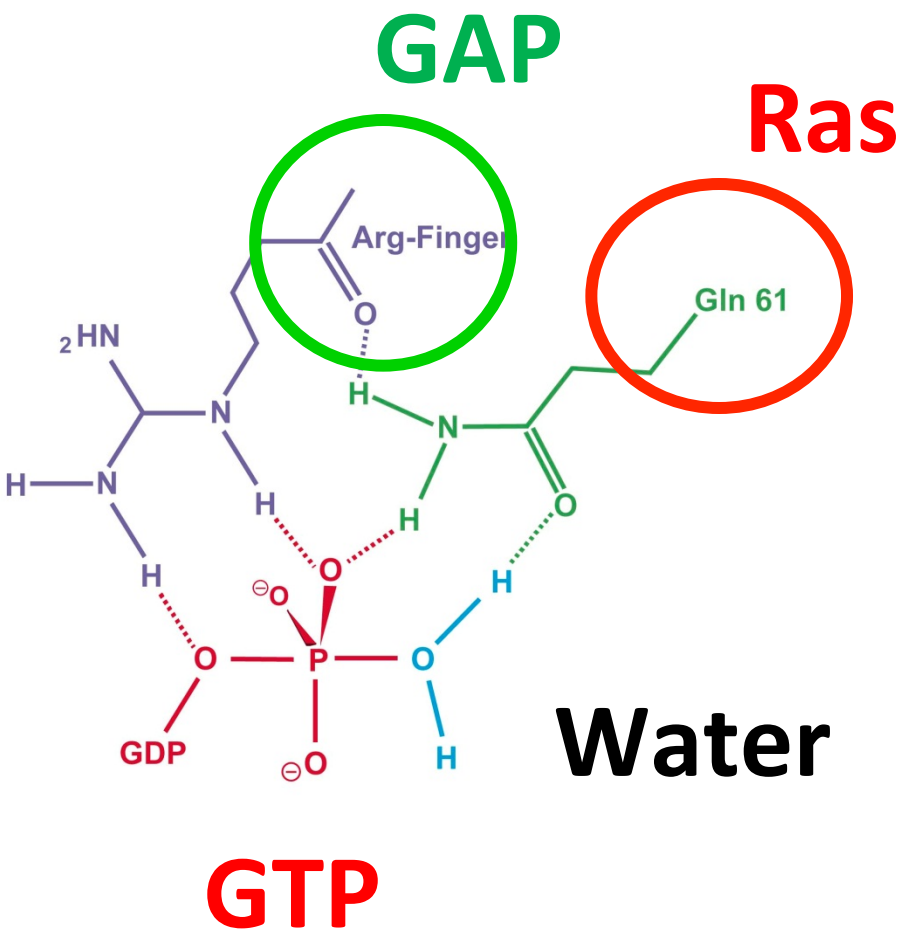
The Ras Pathway



How GAP turns Ras off

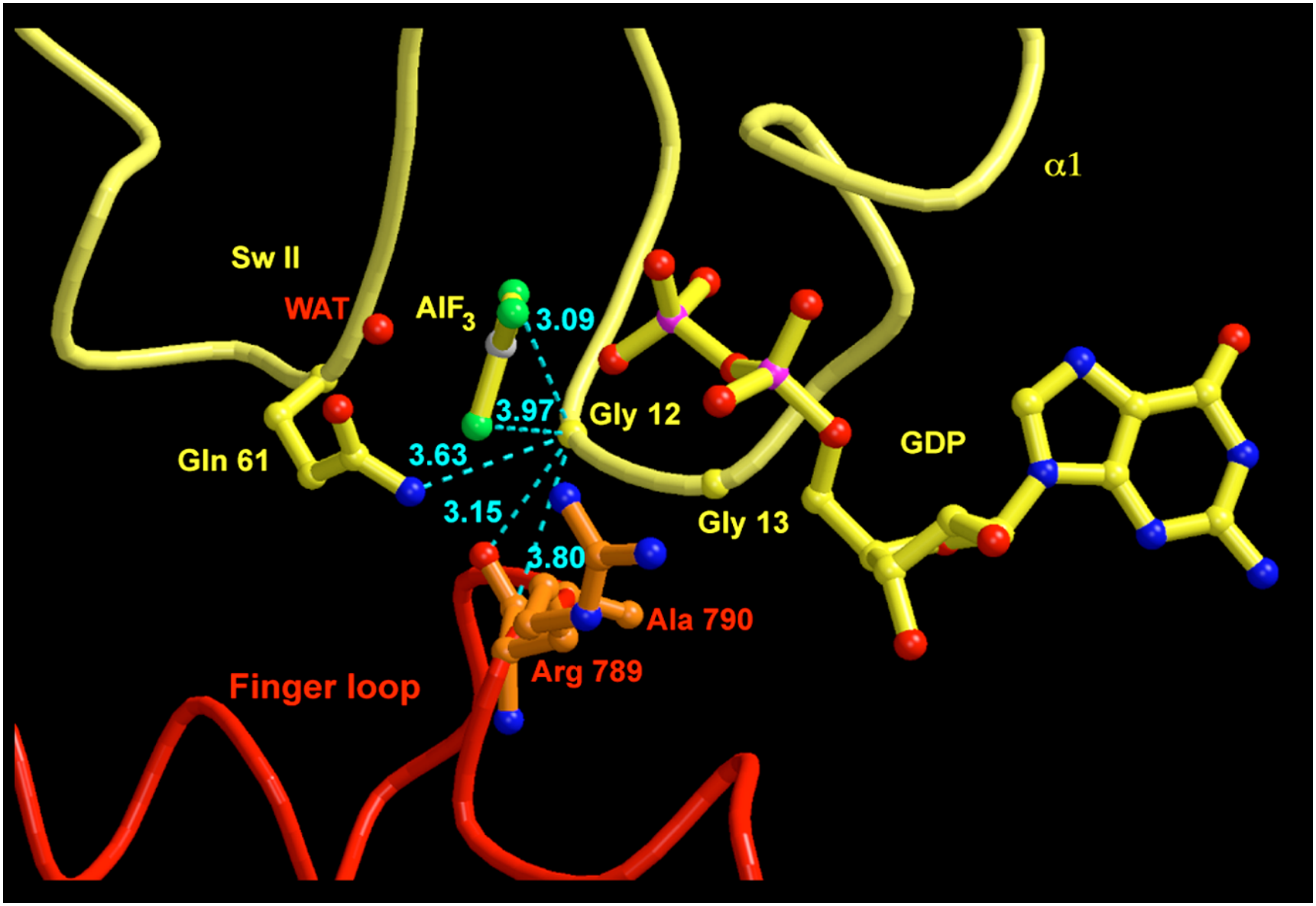


GAP domain from neurofibromin



Fred Wittinghofer http://www.imprs-cb.mpg.de

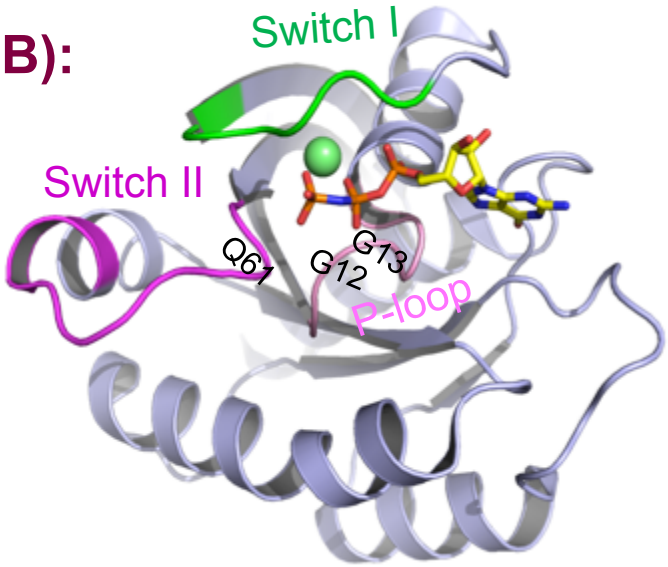
Mutations at position 12 distort the GTPase site



RAS Structural Biology

Structures available in Protein Data Bank (PDB):

- HRAS: 120 structures
- KRAS: 36 structures
- NRAS: 1 structure



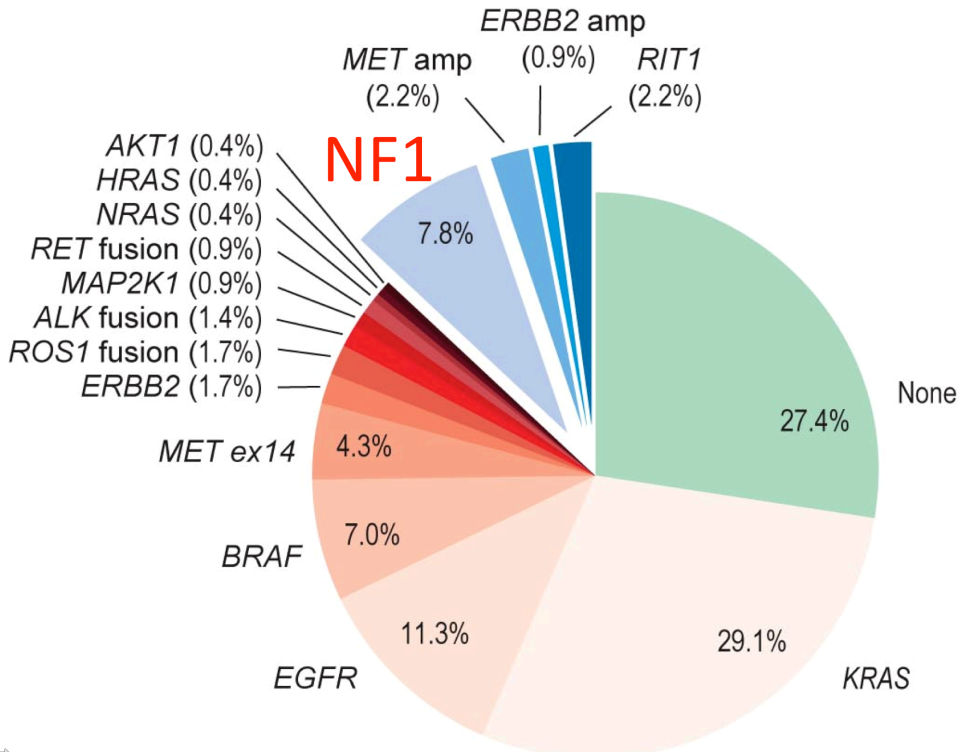
KRAS bound to GTP analog

PDB code: 3GFT

Challenges to targeting RAS cancers

- No structure of KRAS mutants with any effector or regulator.
- No structural insights about how RAS activates Raf kinase.
- No structural information on full-length processed RAS.
- No structural information on full-length Raf – free or in complex with RAS.

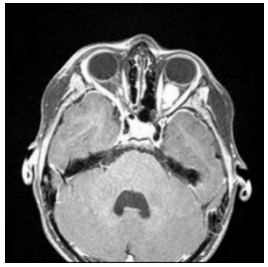
Frequent loss of Neurofibromin in lung adenocarcinoma

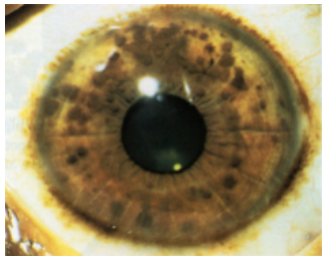


Dr Eric Collisson, TCGA

Neurofibromatosis 1 (NF1)

- Autosomal Dominant, sporadic or familial transmission
- Prevalence 1 in 3500





Dermal or plexiform

Optic nerve tumor

Lisch nodules

Osseous lesion

Cell, Vol. 62, 599–608, August 10, 1990, Copyright © 1990 by Cell Press

The Neurofibromatosis Type 1 Gene Encodes a Protein Related to GAP

Gangfeng Xu,* Peter O'Connell,* David Viskochil,† Richard Cawthon,* Margaret Robertson,* Melanie Culver,* Diane Dunn,* Jeff Stevens,* Ray Gesteland,* Ray White,* and Robert Weiss*
* Department of Human Genetics
and Howard Hughes Medical Institute
† Department of Pediatrics
University of Utah School of Medicine
Salt Lake City, Utah 84132

Our previous studies provided a 4 kb continuous sequence from overlapping cDNA clones, and predicted a large open reading frame of 1234 amino acids with no apparent N-terminus (Cawthon et al., 1990). Searches of several data bases yielded no significant similarity between the predicted peptide and any protein in the PIR and SwissProt protein data bases, or any six-frame translated nucleic acid sequence in the EMBL and GenBank data bases.

Received 24 July 1990

Cell, Vol. 63, 843–849, November 16, 1990, Copyright © 1990 by Cell Press

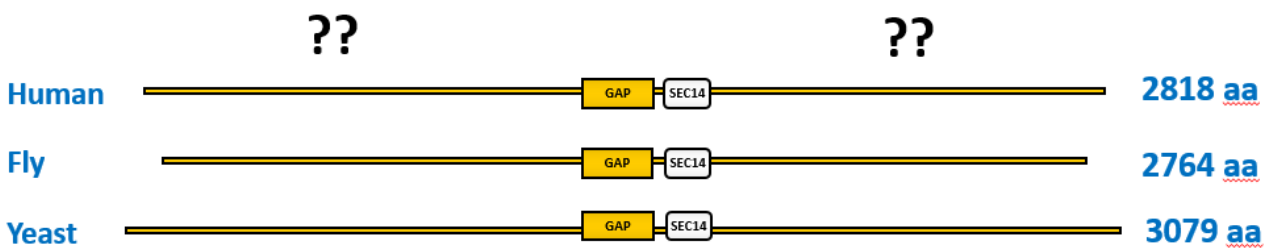
The GAP-Related Domain of the Neurofibromatosis Type 1 Gene Product Interacts with *ras* p21

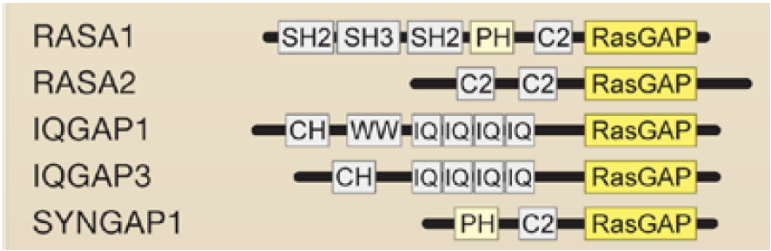
George A. Martin,* David Viskochil,† Gideon Bollag,* Peter C. McCabe,* Walter J. Crosier,* Heinz Haubruck,* Leah Conroy,* Robin Clark,* Peter O'Connell,‡ Richard M. Cawthon,‡ Michael A. Innis,* and Frank McCormick*
* Department of Molecular Biology
Cetus Corporation
Emeryville, California 94608
† Department of Pediatrics
‡ Department of Human Genetics
and Howard Hughes Medical Institute
University of Utah School of Medicine
Salt Lake City, Utah 84132

GTPase stimulation and hence signal termination. According to this hypothesis, oncogenic *ras* p21 mutants, which retain their ability to bind GAP (Vogel et al., 1988), signal constitutively because signal termination is defective. Alternatively, it is possible that *ras* p21 (in its GTP-bound form) interacts with as yet unidentified effector proteins and that GAP-mediated GTP hydrolysis is not coupled directly to effector function. This appears to be the case for Saccharomyces cerevisiae, where *RAS* proteins (in their GTP-bound states) activate an effector (adenylyl cyclase; Toda et al., 1985; Broek et al., 1985) and are subsequently converted to their inactive GDP-bound states by putative GAPs encoded by the *IRA* genes (Tanaka et al., 1989). While it remains possible that the interaction be-

Received October 2, 1990

Neurofibromin



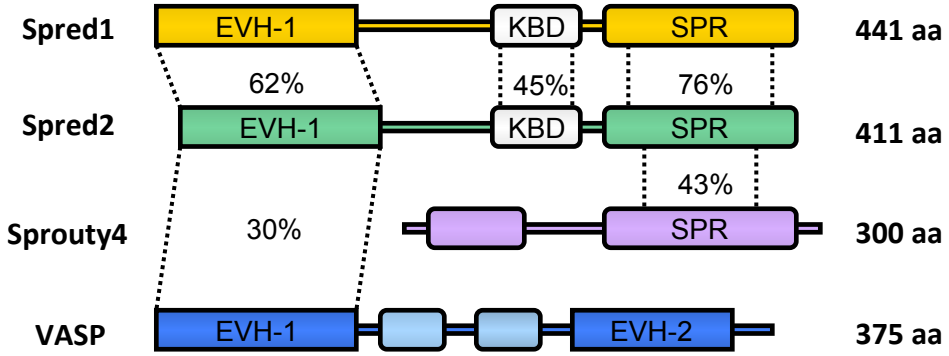


Legius Syndrome

Multiple café-au-lait macules

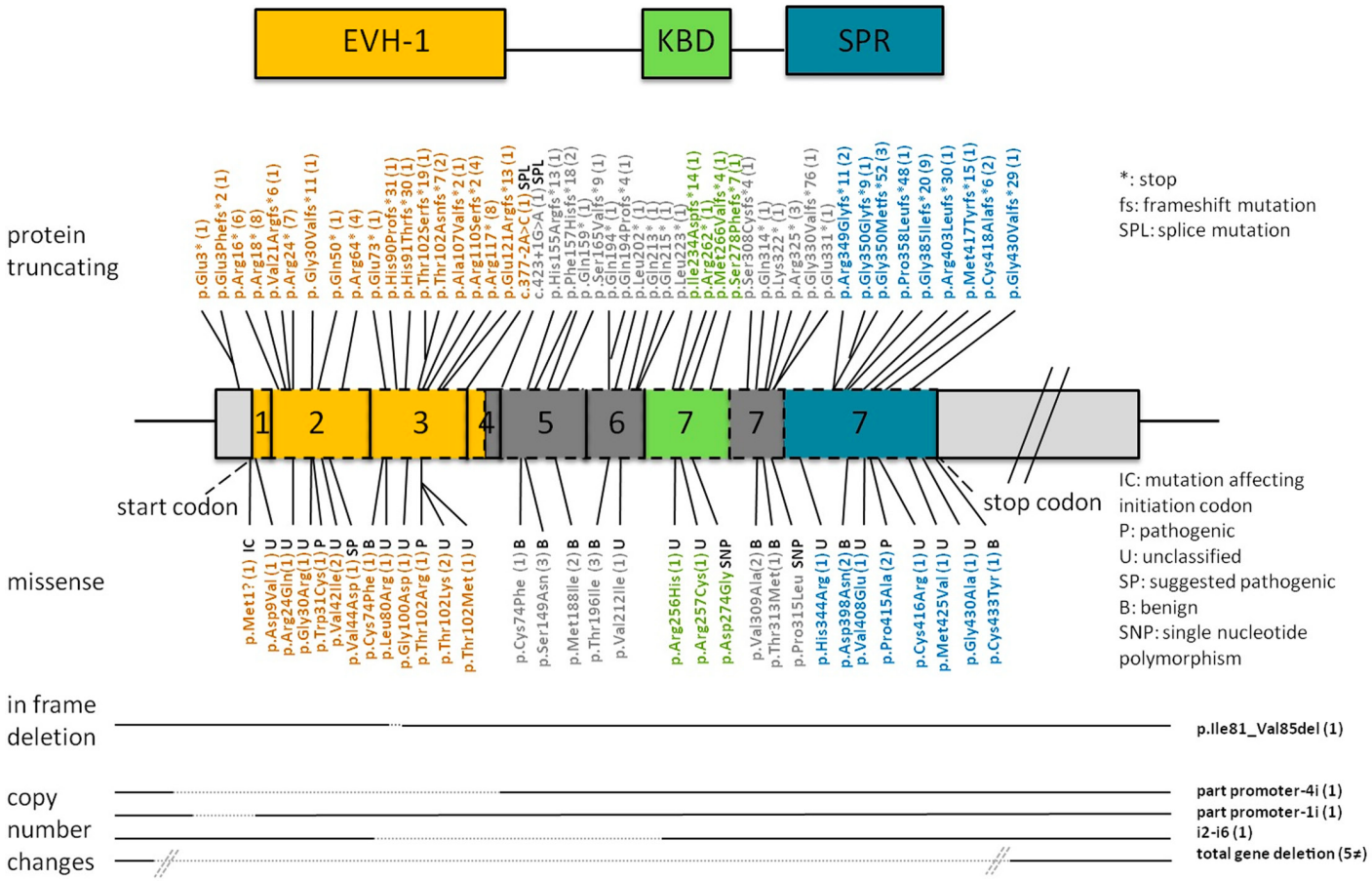
Axillary freckling

Macrocephaly
Noonan-like dysmorphy
Learning disabilities / ADHD
developmental delays

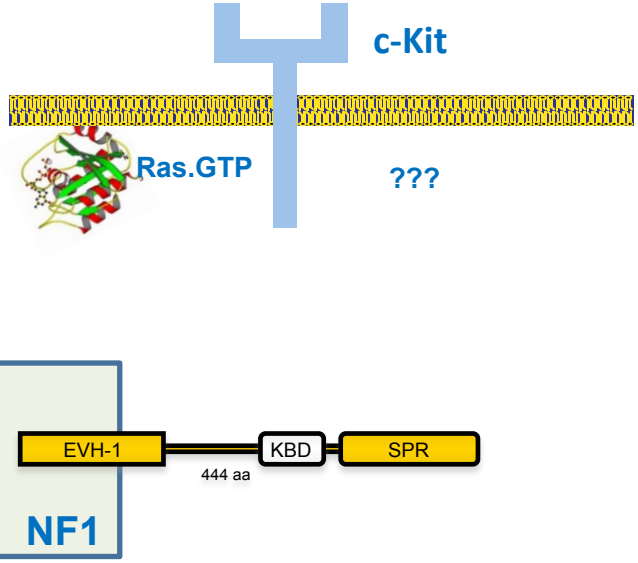


EVH domain: 'ENA/VASP Homology proteins'

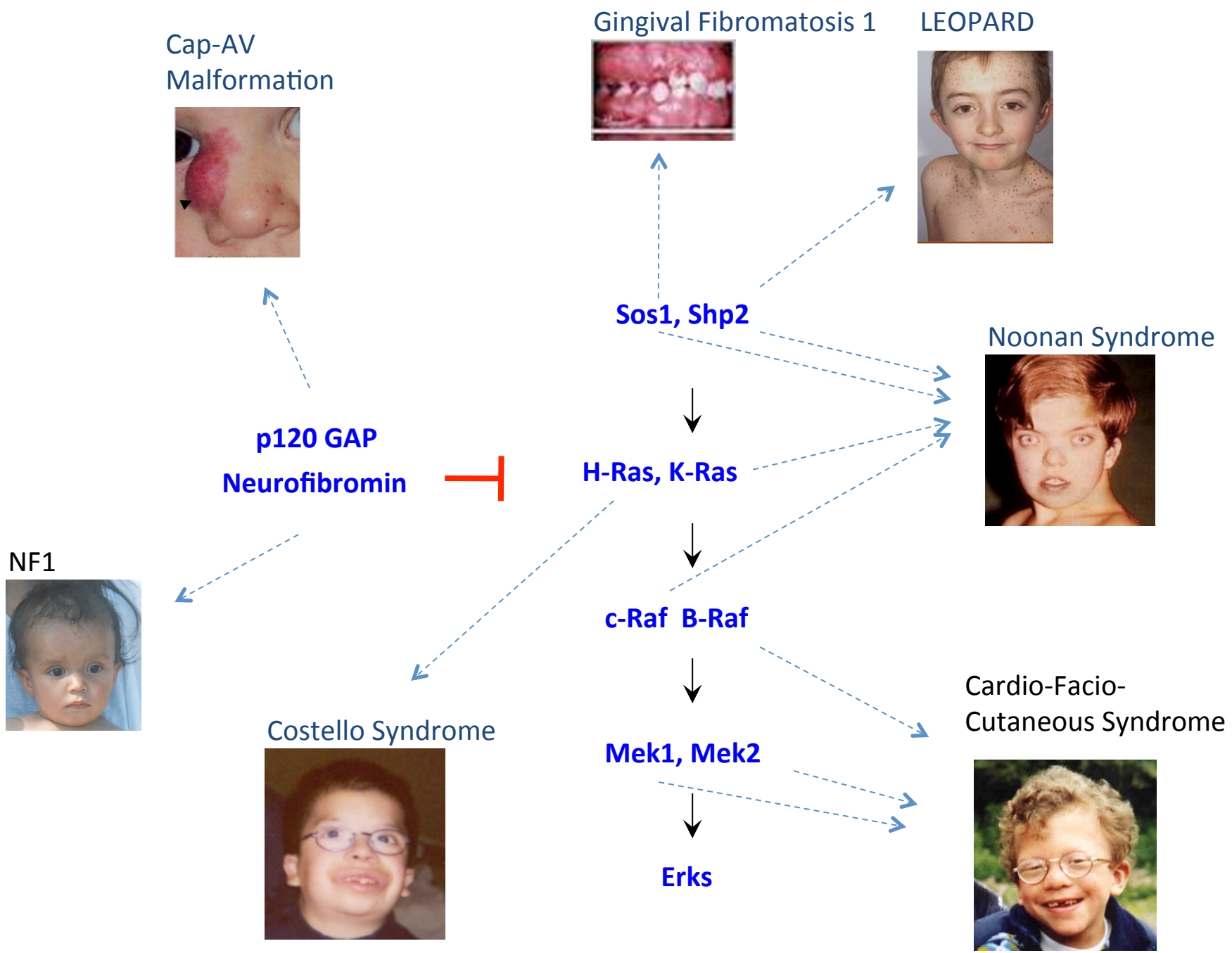
Pathogenic mutations in SPRED1 in Legius syndrome



SPRED recruits NF1 to Ras.GTP



Stowe, IB, Mercado, EL, Stowe, TR, Bell, EL, Oses-Prieto, JA, Hernández, H, Burlingame, AL and McCormick, F. A shared molecular mechanism underlies the human rasopathies, Legius syndrome and Neurofibromatosis-1, 2012 Genes Dev. 2012 Jul 1; 26(13): 1421-6.


Cap-AV
Malformation
Gingival Fibromatosis 1
LEOPARD
Sos1, Shp2
Noonan Syndrome
p120 GAP
Neurofibromin
H-Ras, K-Ras
NF1
c-Raf B-Raf
Costello Syndrome
Cardio-Facio-
Cutaneous Syndrome
Mek1, Mek2
Erks

Incidence of KRAS mutations in 3 diseases

	G12C	G12D	G12V	G13D
Colorectal	6,300	22,000	12,600	11,250
Lung	22,000	9,520	11,900	1,190
Pancreas	1,200	19,000	12,000	1,000
Total	29,500	50,520	36,500	13,440

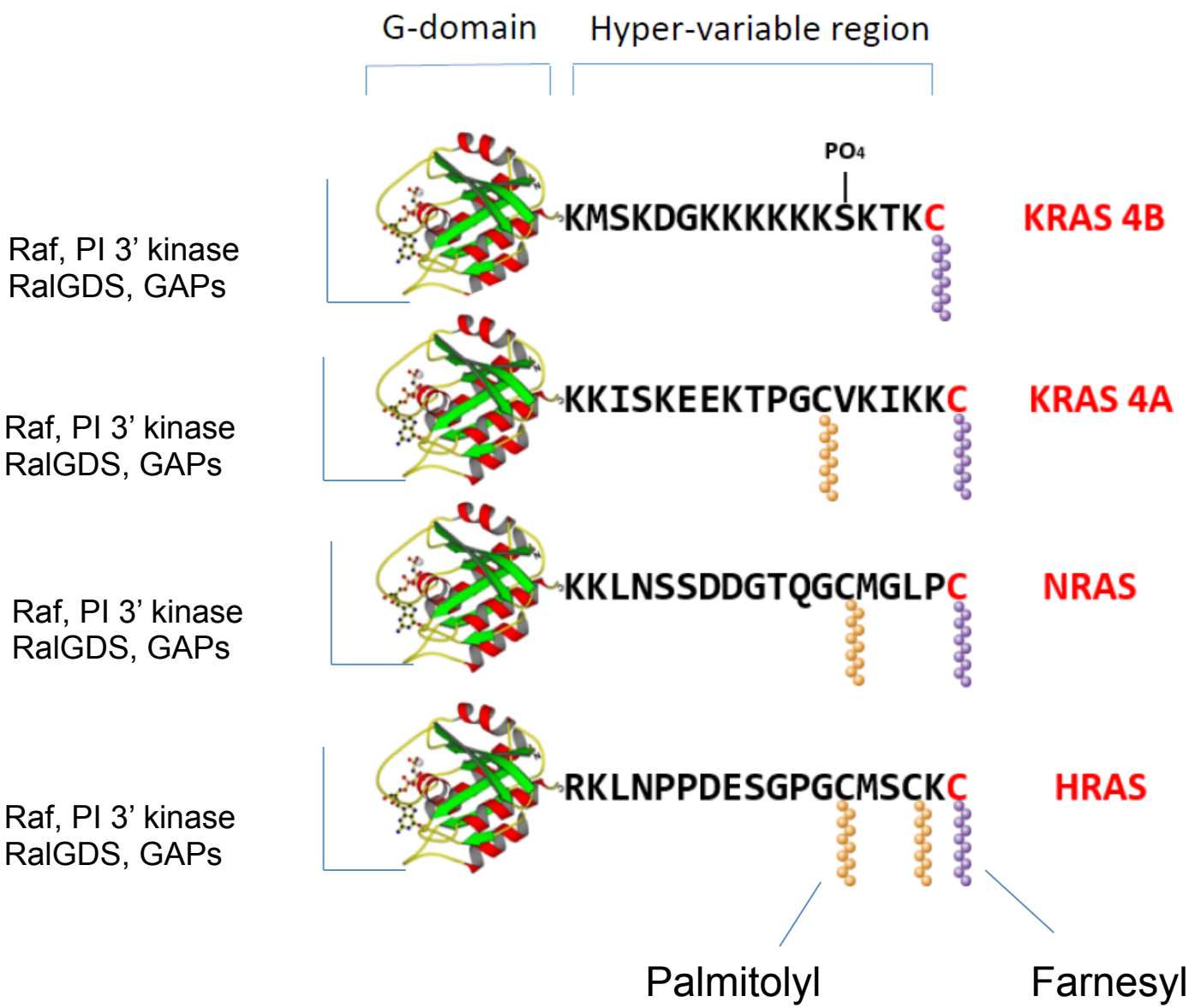
Distinct biological and clinical properties of KRAS alleles

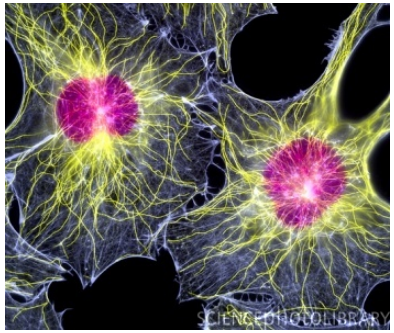
-KRAS G12V, G12C: worse clinical outcome than G12D
(Al-Mulla et al; Andreyev et al; Vega et al; Keohavong et al)

-KRAS G12D: elevated PI 3' kinase, MAPK signaling

-KRAS G12C, G12V: elevated RalGDS signaling
(Ihle et al, 2012)

-KRAS G13D: respond to Cetuximab: G12 mutants do not….
(de Roock et al, 2010)

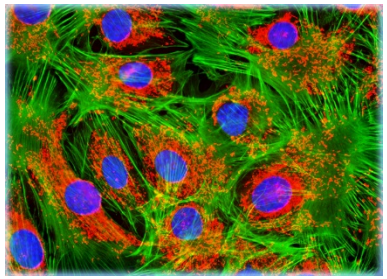

G-domain
Hyper-variable region
PO4
KMSKDGKKKKKKSKTKC
KRAS 4B
Raf, PI 3' kinase
RalGDS, GAPs
KKISKEEKTPGCVKIKKC
KRAS 4A
Raf, PI 3' kinase
RalGDS, GAPs
KKLNSSDDGTQGCMGLPC
NRAS
Raf, PI 3' kinase
RalGDS, GAPs
RKLNPPDESGPGCMSCKC
HRAS
Raf, PI 3' kinase
RalGDS, GAPs
Palmitolyl
Farnesyl

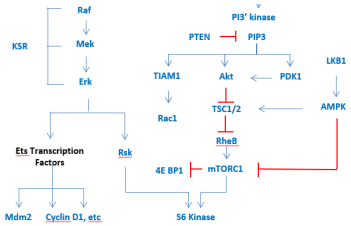


Rasless MEFs

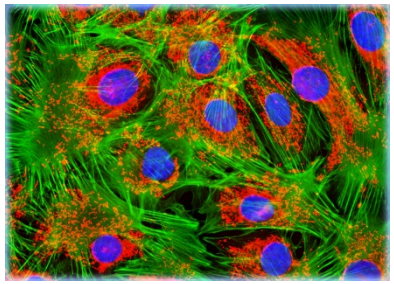
Drosten M, Dhawahir A, Sum EY, Urosevic J, Lechuga CG, Esteban LM, Castellano E, Guerra C, Santos E, **Barbacid M**.
EMBO J. 2010

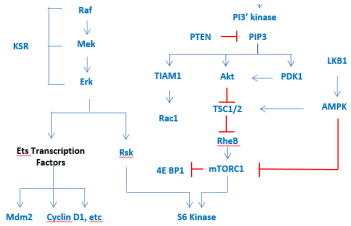
HRAS



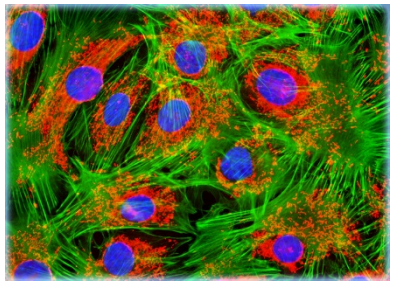


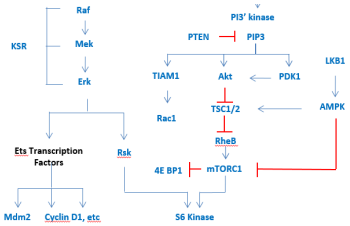
NRAS



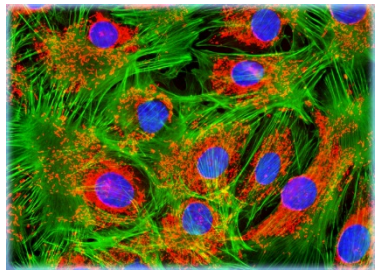


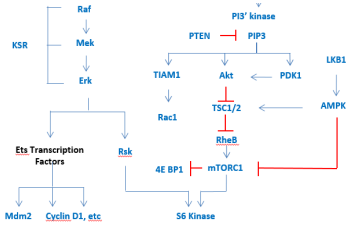
KRAS4A





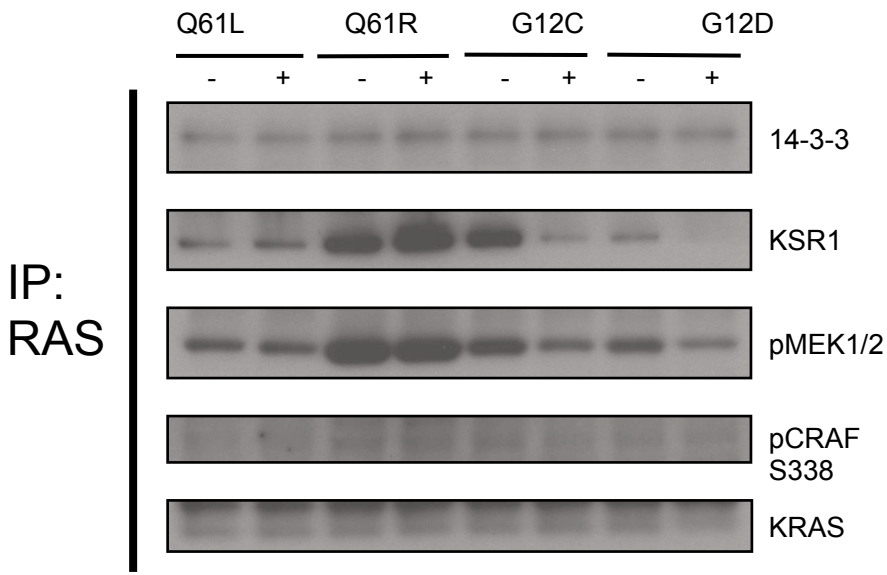
KRAS4B

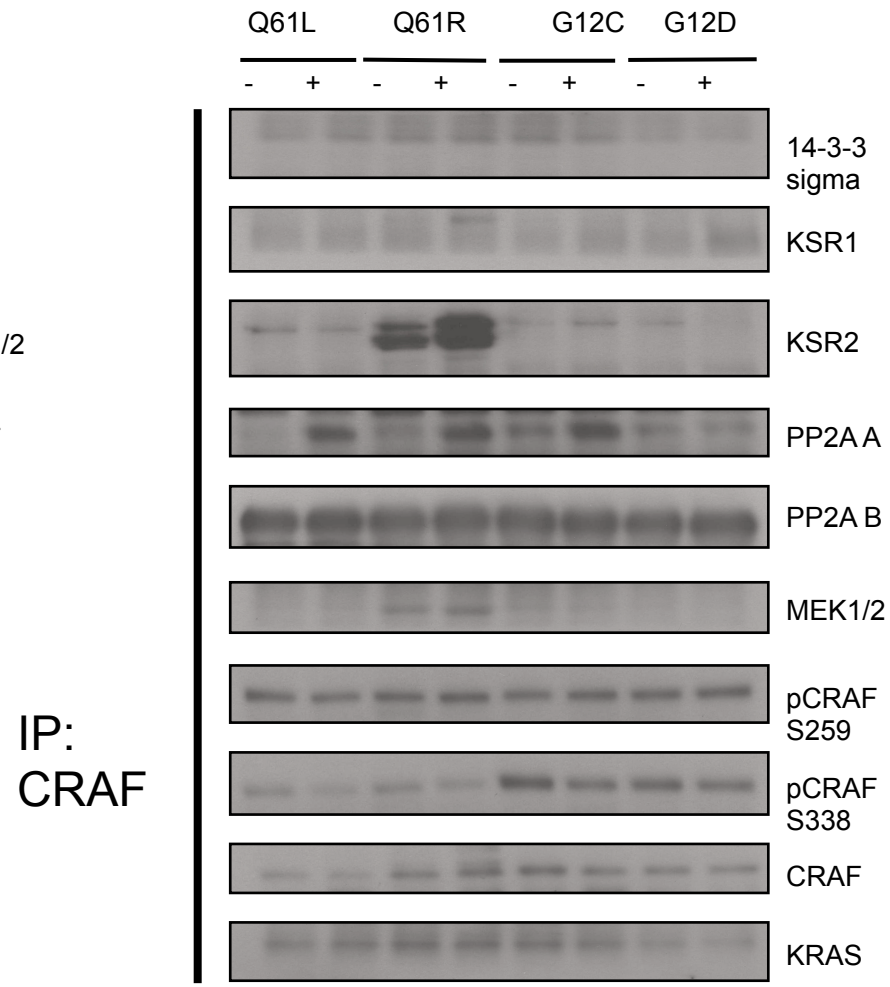




Cameron Pitt

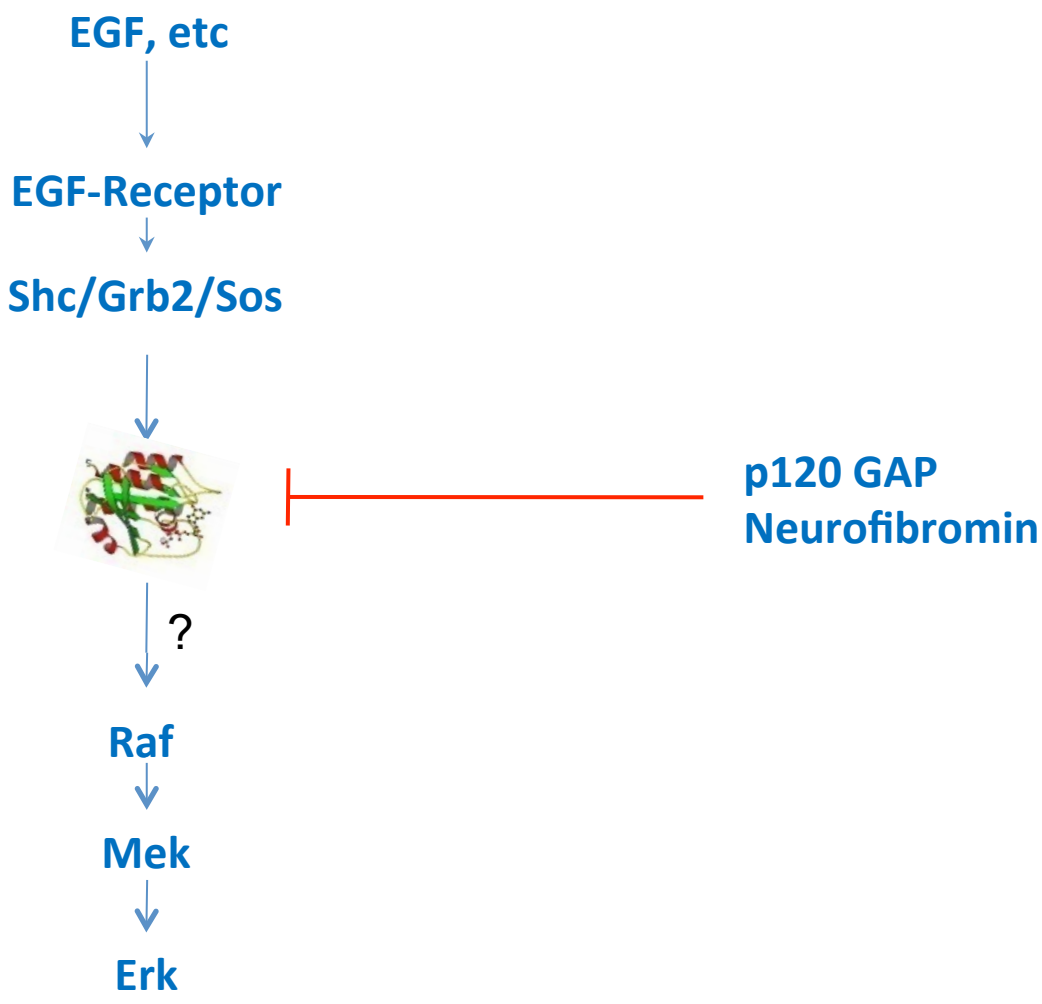
Distinct signaling complexes in different oncogenic mutants



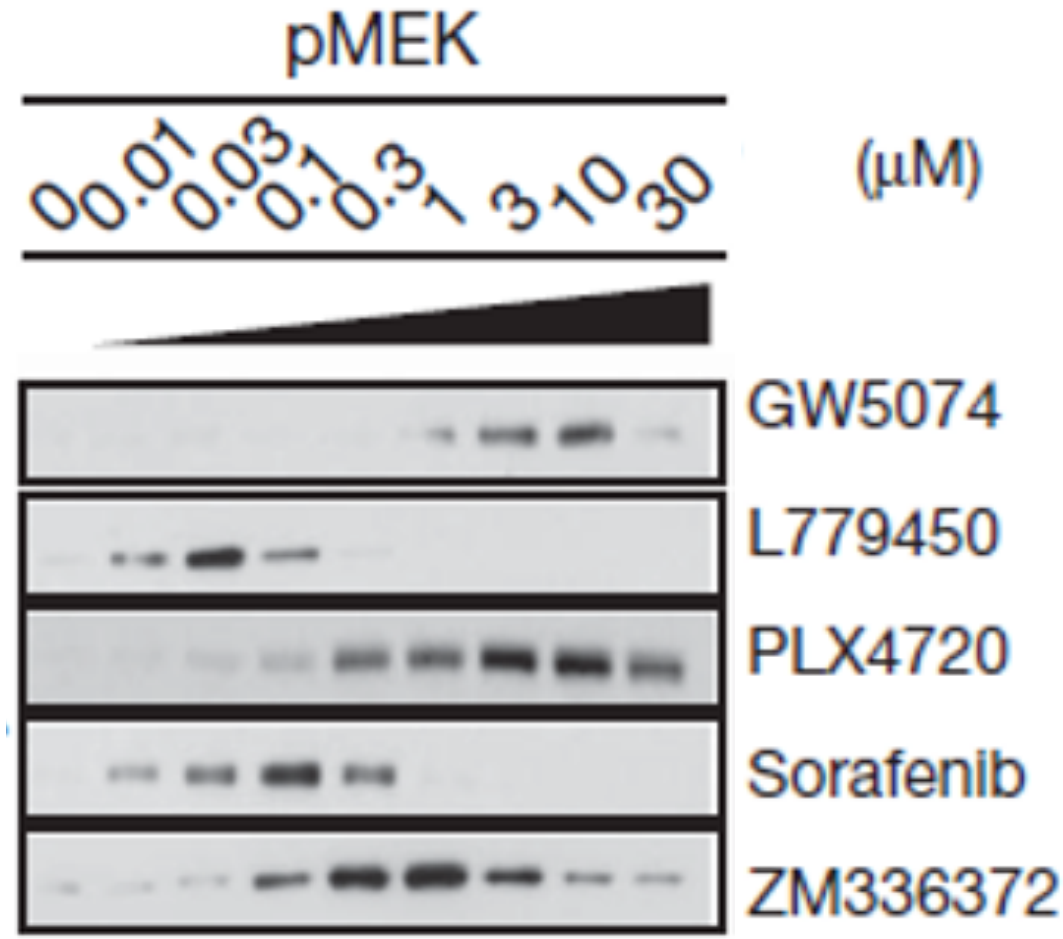


- : U73343 non-targeting analog
+: U73122 PLC inhibitor

The Ras pathway, circa 1991

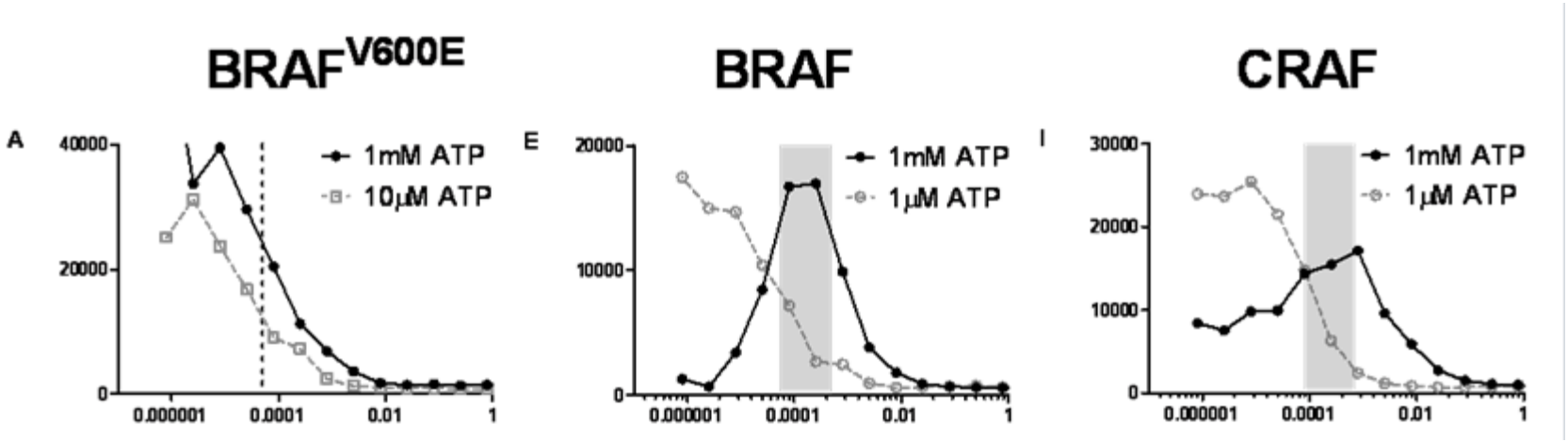


RAF inhibitors activate RAF in cells with mutant KRAS!



Neal Rosen et al

RAF inhibitors activate pure RAF in vitro



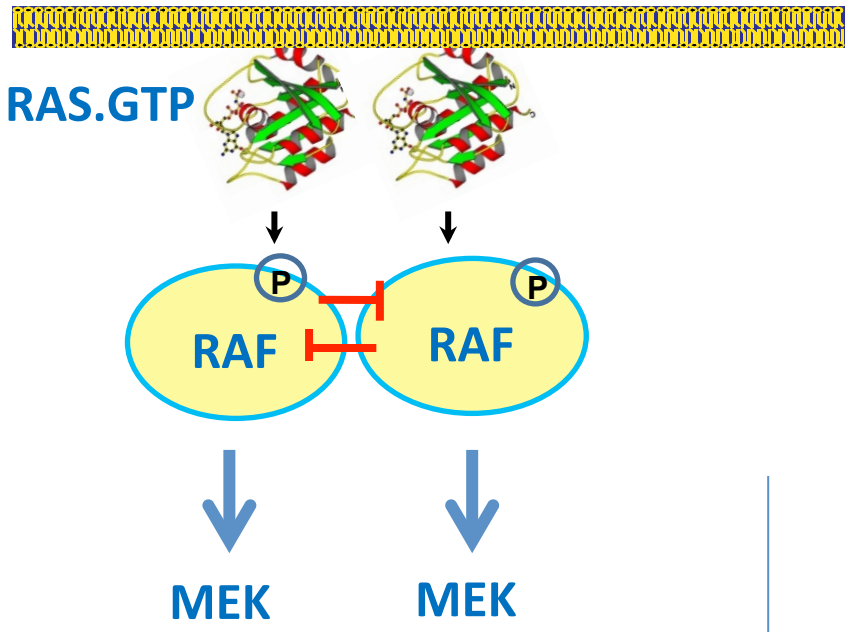
Raf inhibitor →

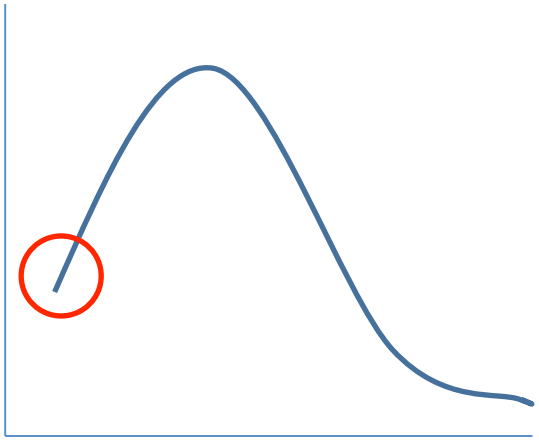
RAF inhibitors activate the MAPK pathway by relieving inhibitory auto-phosphorylation

Matthew Holderfield[1], Hanne Merritt[1], John Chan[1], Marco Wallroth[1], Laura Tandeske[1], Huili Zhai[2], John Tellew[3], Steve Hardy[1], Mohammad Hekmat-Nejad[1], Darrin Stuart[1], Frank McCormick[4], Tobi Nagel[1]

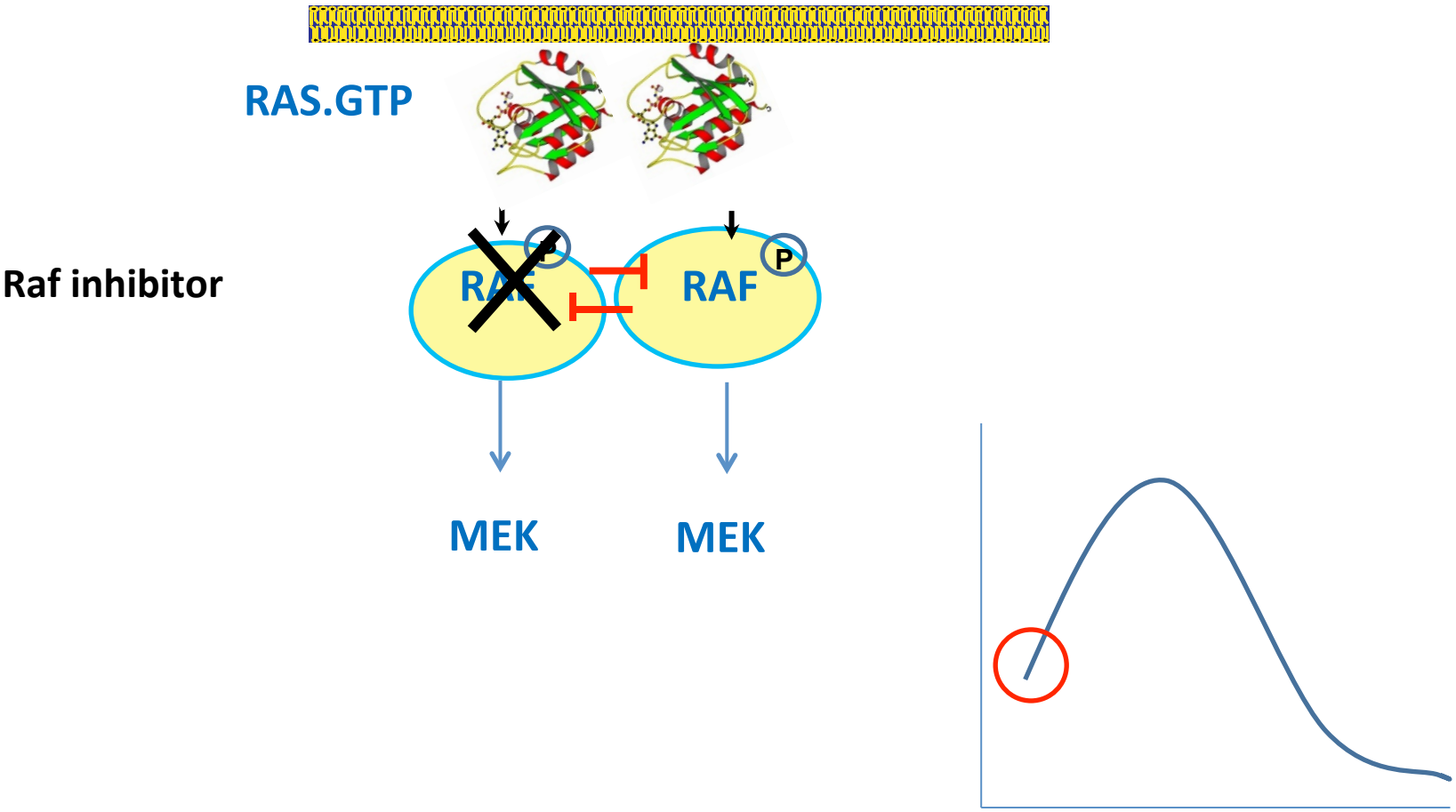
Cancer Cell, *2013*

Activation of Raf kinase by Raf inhibitors

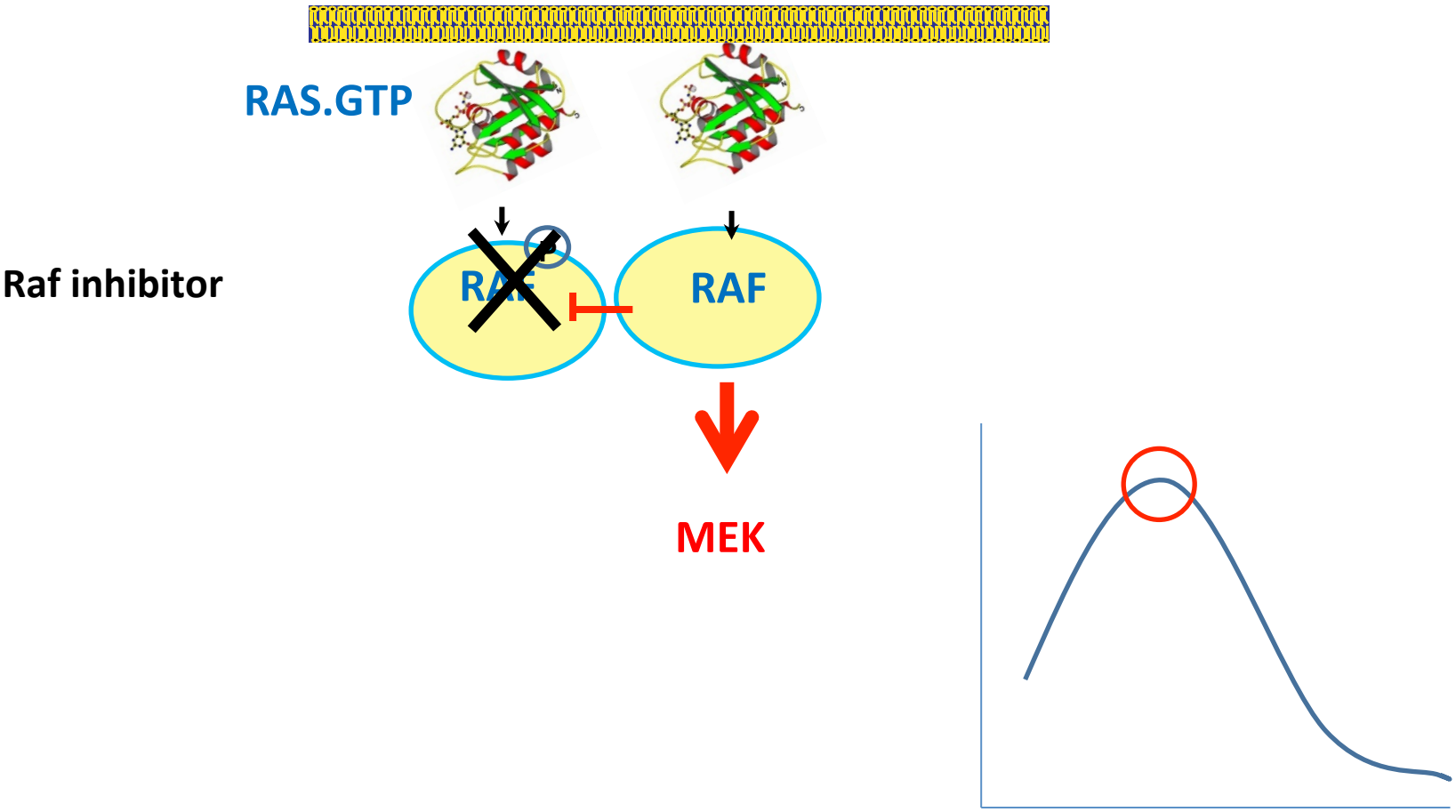




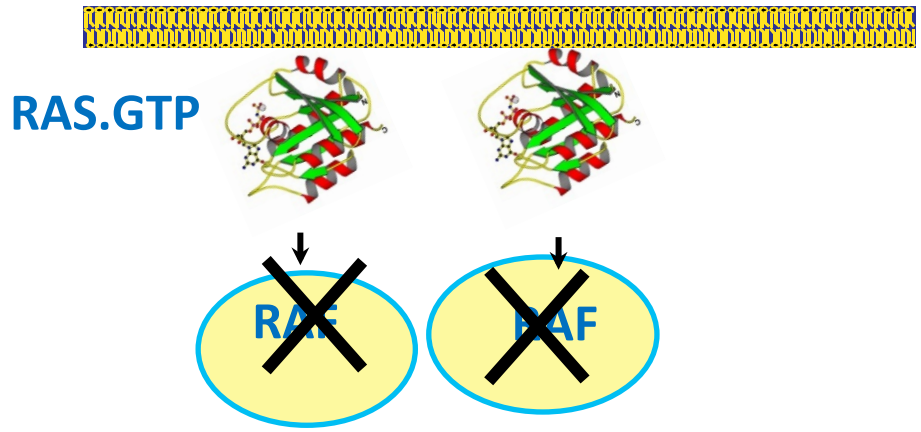
Activation of Raf by Raf inhibitors



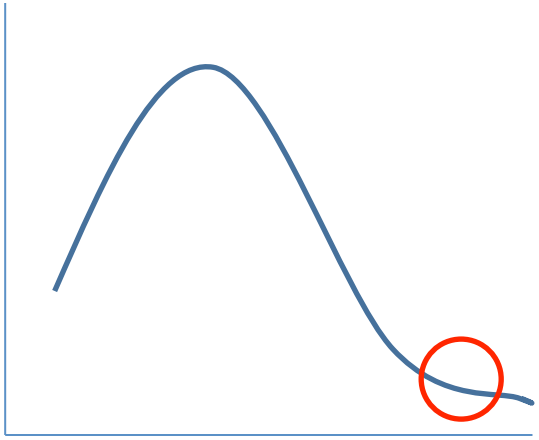
Activation of Raf by Raf inhibitors



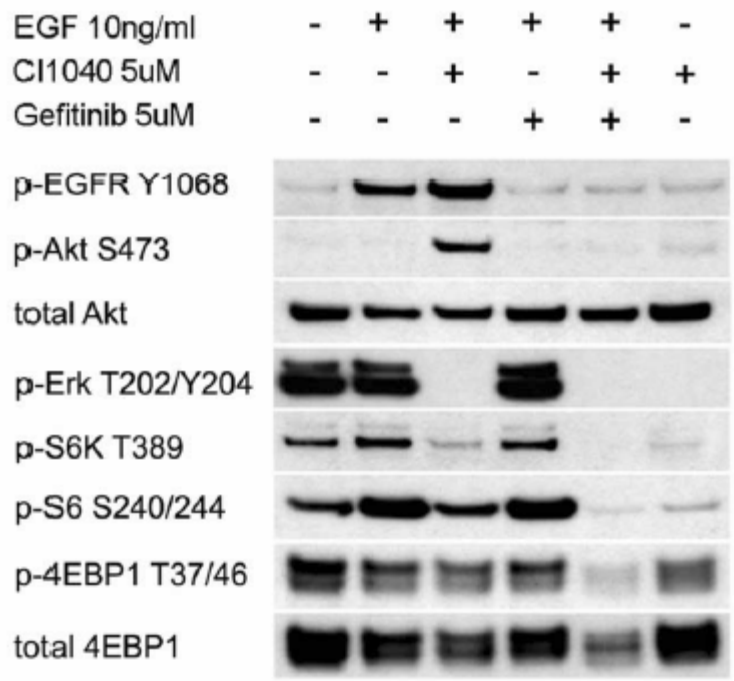
Activation of Raf by Raf inhibitors



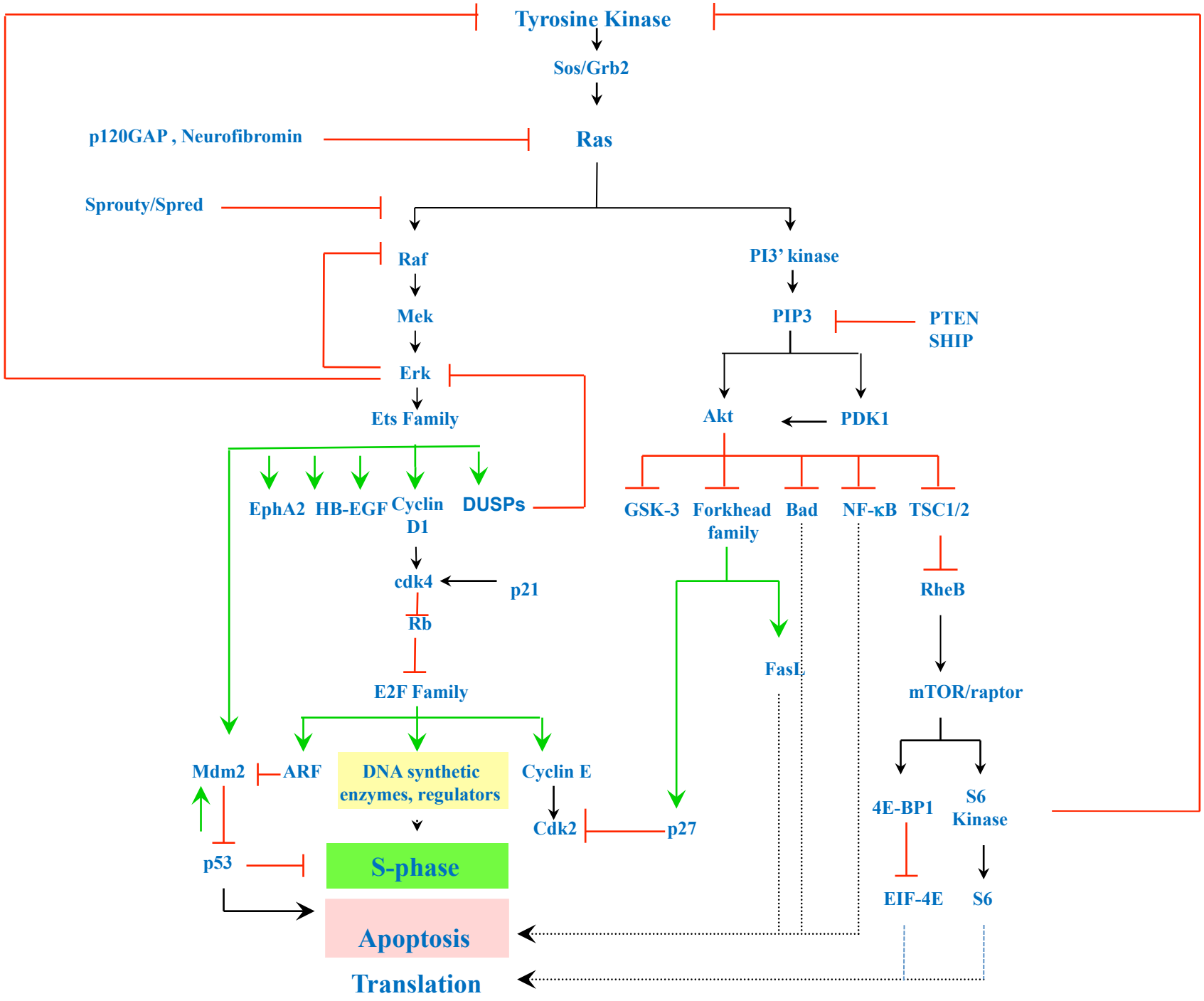
More Raf inhibitor



Up-regulation of EGF signaling following MEK inhibition



Mirzoeva OK, Das D, Heiser LM, Bhattacharya S, Siwak D, Gendelman R, Bayani N, Wang NJ, Neve RM, Guan Y, Hu Z, Knight Z, Feiler HS, Gascard P, Parvin B, Spellman PT, Shokat KM, Wyrobek AJ, Bissell MJ, McCormick F, Kuo WL, Mills GB, Gray JW, Korn WM.
Cancer Res. 2009 Jan 15;69(2):565-72.

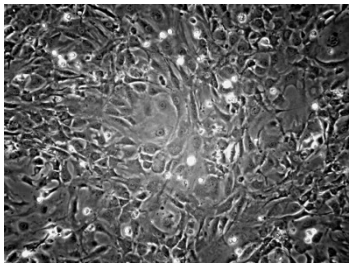

Tyrosine Kinase
Sos/Grb2
p120GAP , Neurofibromin
Ras
Sprouty/Spred
Raf
Mek
Erk
Ets Family
PI3' kinase
PIP3
PTEN
SHIP
Akt
PDK1
EphA2
HB-EGF
Cyclin D1
DUSPs
GSK-3
Forkhead family
Bad
NF-κB
TSC1/2
cdk4
p21
Rb
E2F Family
RheB
FasL
mTOR/raptor
Mdm2
ARF
DNA synthetic enzymes, regulators
Cyclin E
Cdk2
p27
4E-BP1
S6 Kinase
p53
S-phase
EIF-4E
S6
Apoptosis
Translation

RAS mutations in human cancer

Pancreas	95%	KRAS
Colorectal	45%	KRAS
Lung	35%	KRAS
AML	30%	NRAS
Melanoma	15%	NRAS
Bladder Cancer	5%	HRAS

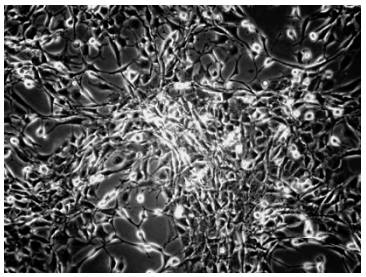
HRAS and KRAS transformed NIH3t3 cells show similar levels of active Erk and Akt

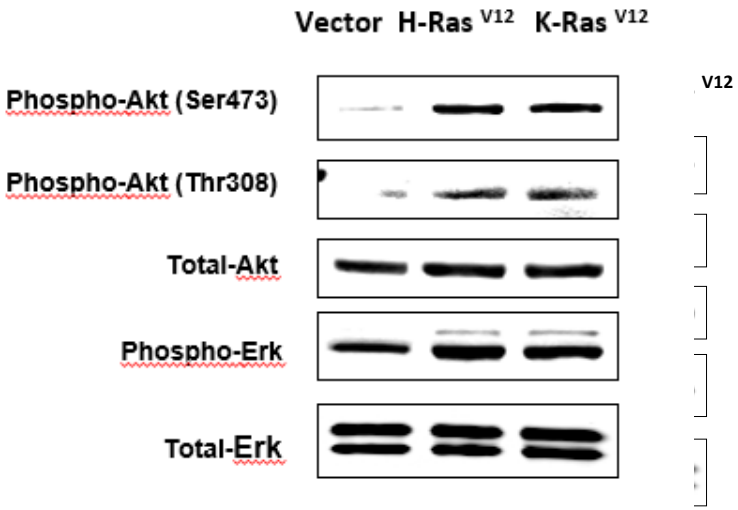
Vector Control

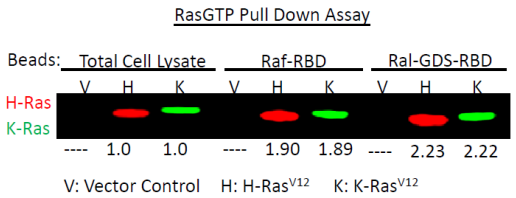


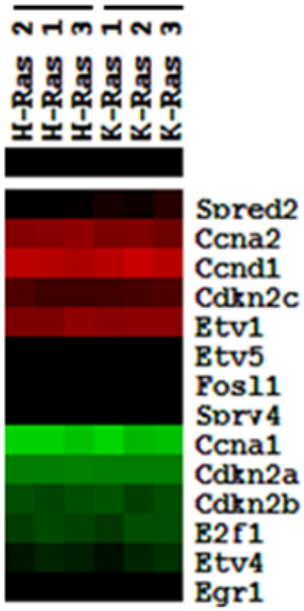
H-Ras^{V12}

K-Ras^{V12}



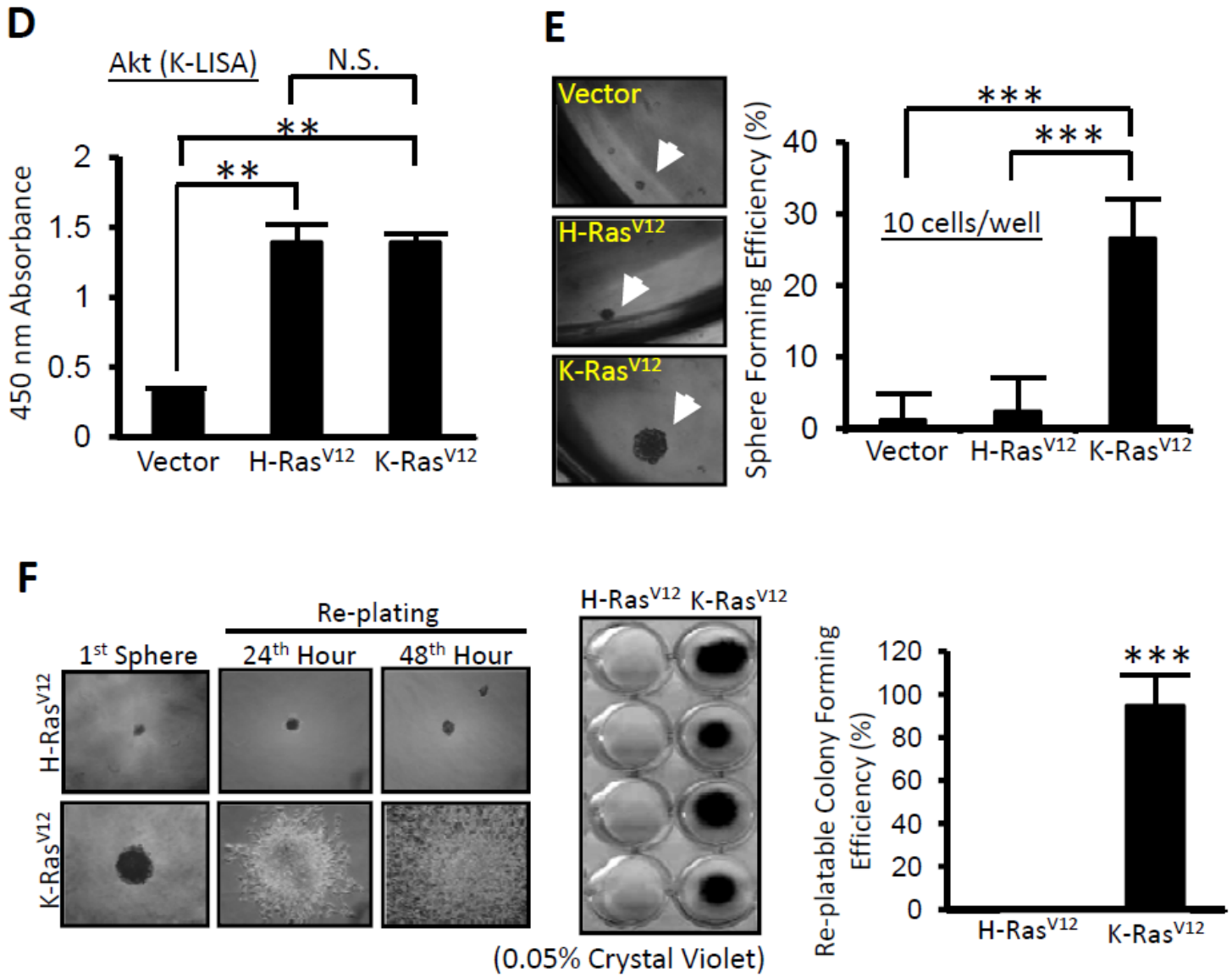




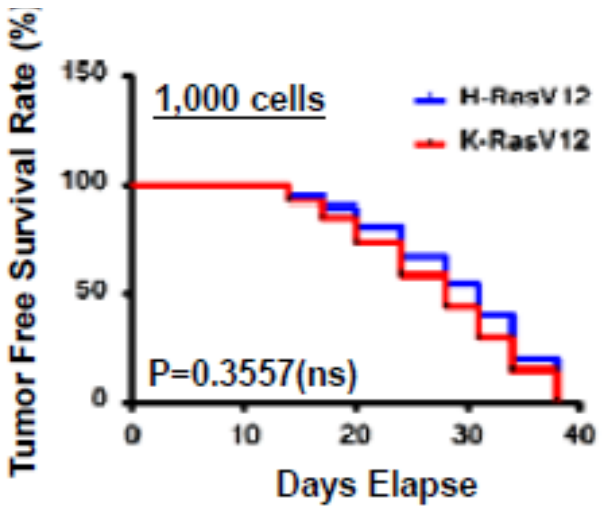


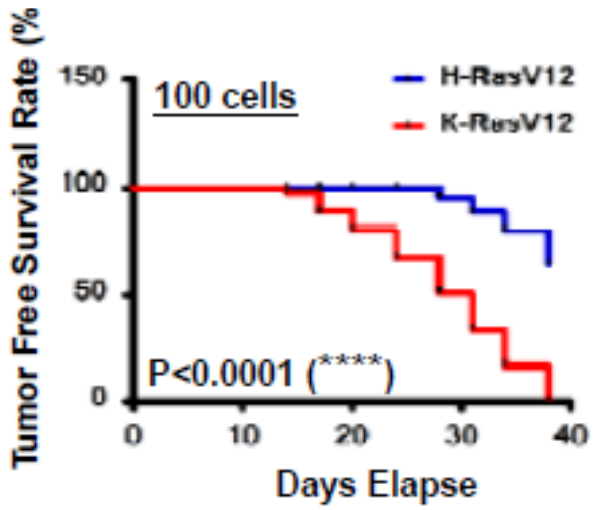
MAPK target genes

KRAS, but not HRAS, confers a stem-cell phenotype

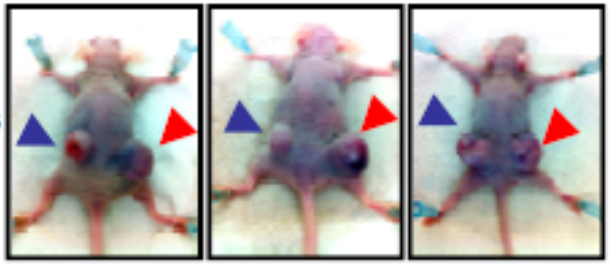


KRAS, but not HRAS, confers a stem-cell phenotype

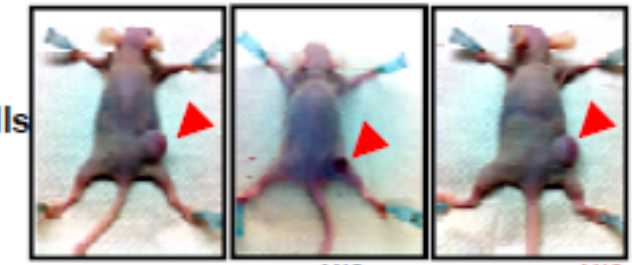




10^3 cells



10^2 cells



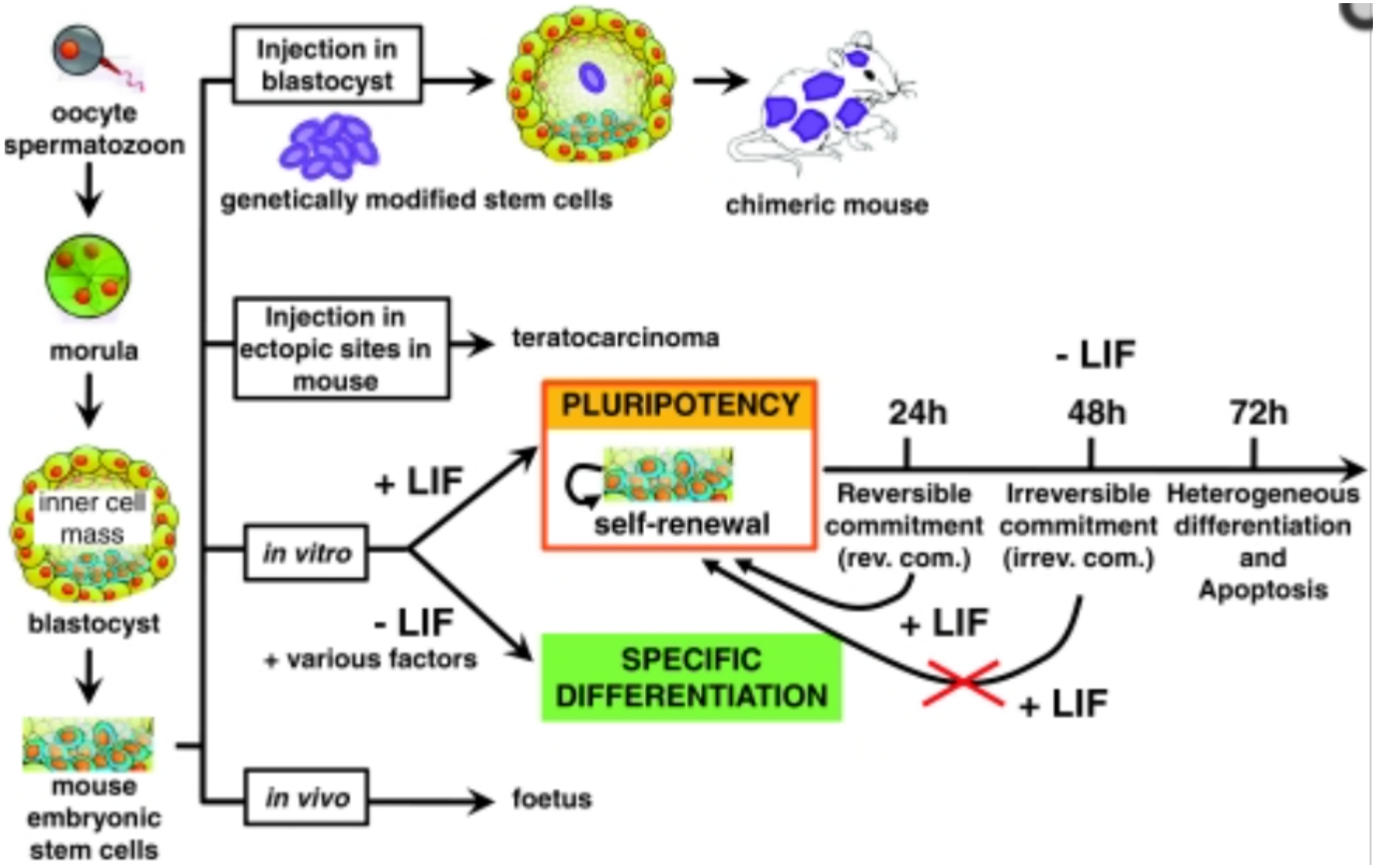
Left: H-Ras^{V12} Right: K-Ras^{V12}

Genes up-regulated by KRAS (G12V) vs HRAS (G12V)

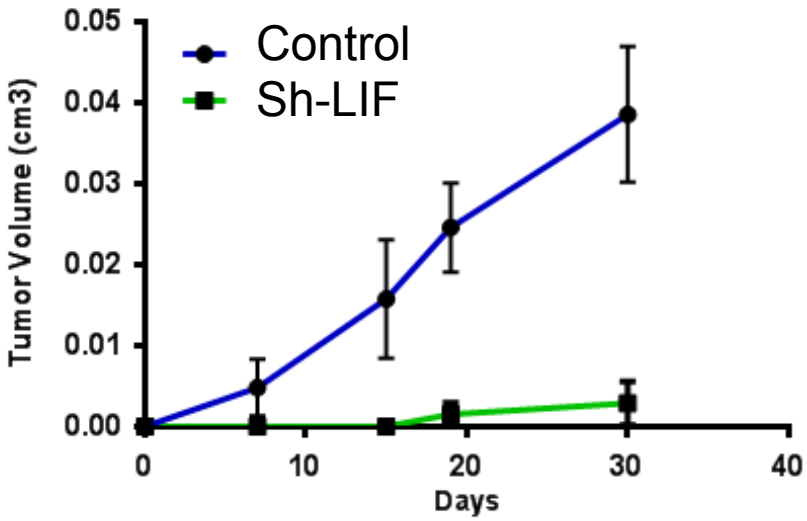
Leukemia Inhibitory Factor	Maintains stem cell in undifferentiated state
Notch 2	Key role in development
C-Myc	Everything
Twist 2	Promotes EMT, blocks differentiation
Abcb1a	Multiple drug resistance

Genes down-regulated by KRAS (G12V) vs HRAS (G12V)

Fzd 8	Drives non-canonical *wnt* signaling
Gli2	Mediates sonic hedgehog signaling

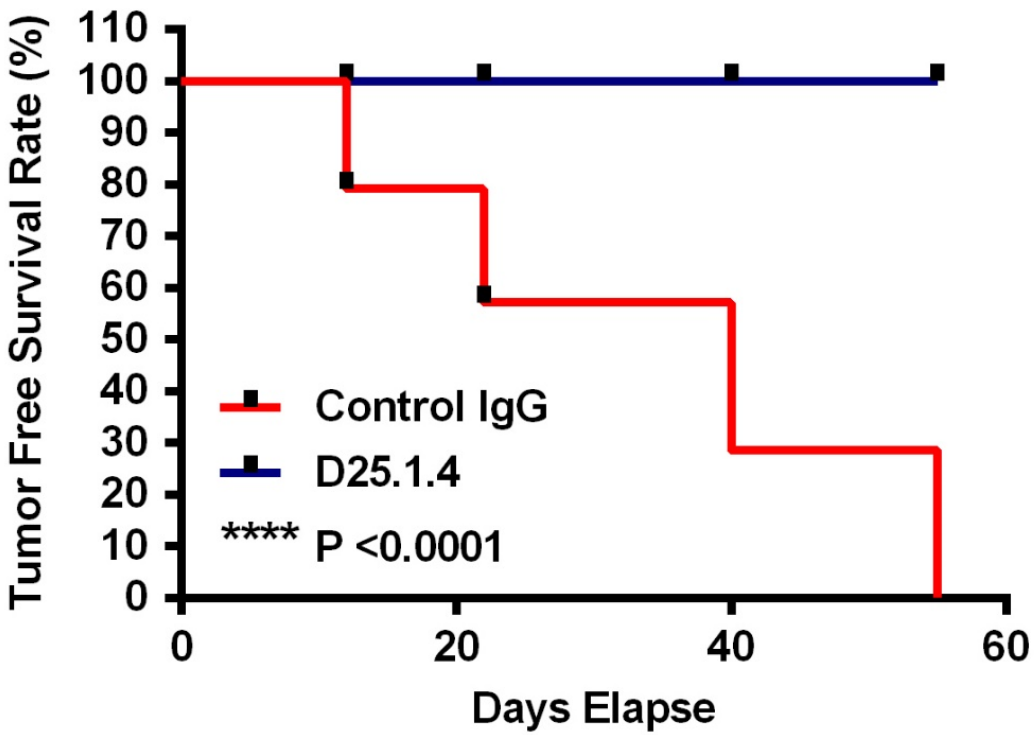

oocyte
spermatozoon
morula
inner cell mass
blastocyst
mouse embryonic stem cells
Injection in blastocyst
genetically modified stem cells
chimeric mouse
Injection in ectopic sites in mouse
teratocarcinoma
in vitro
+ LIF
- LIF
+ various factors
PLURIPOTENCY
self-renewal
SPECIFIC DIFFERENTIATION
- LIF
24h
48h
72h
Reversible commitment (rev. com.)
Irreversible commitment (irrev. com.)
Heterogeneous differentiation and Apoptosis
+ LIF
+ LIF
in vivo
foetus

LIF knock-down suppresses tumor initiation



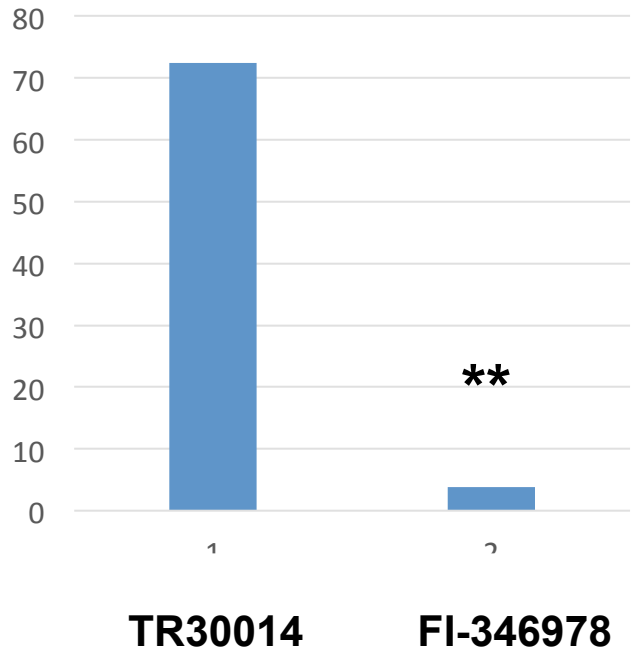
5,000 PANC2 subcutaneously injected in NUDE mice

LIF neutralizing antibodies prevent tumor initiation



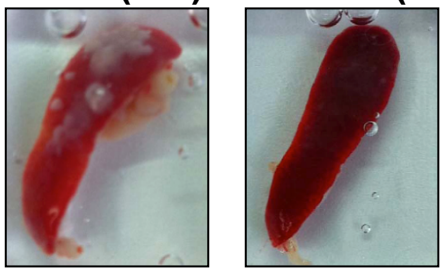
Ab treatment via IP injections given on Day 0 of tumor inoculation

LIF knock-down suppresses tumor initiation and metastasis



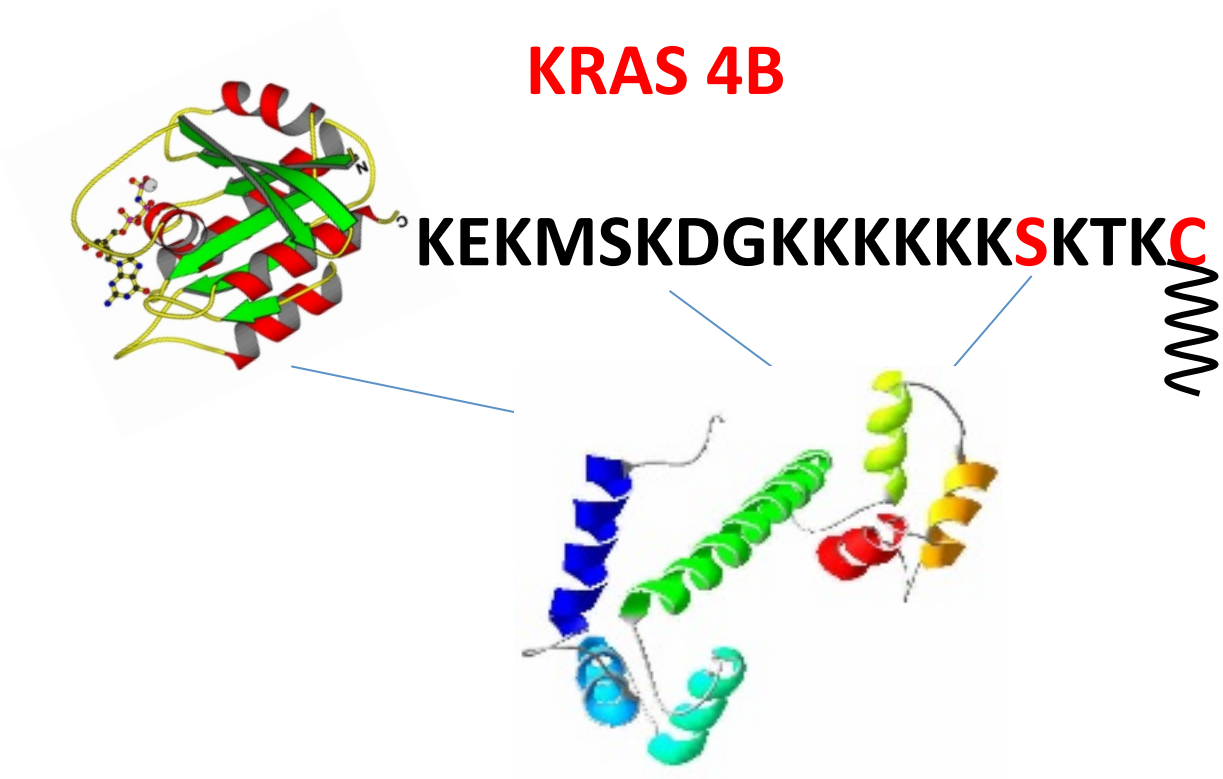
Spleen Macro-Meta.

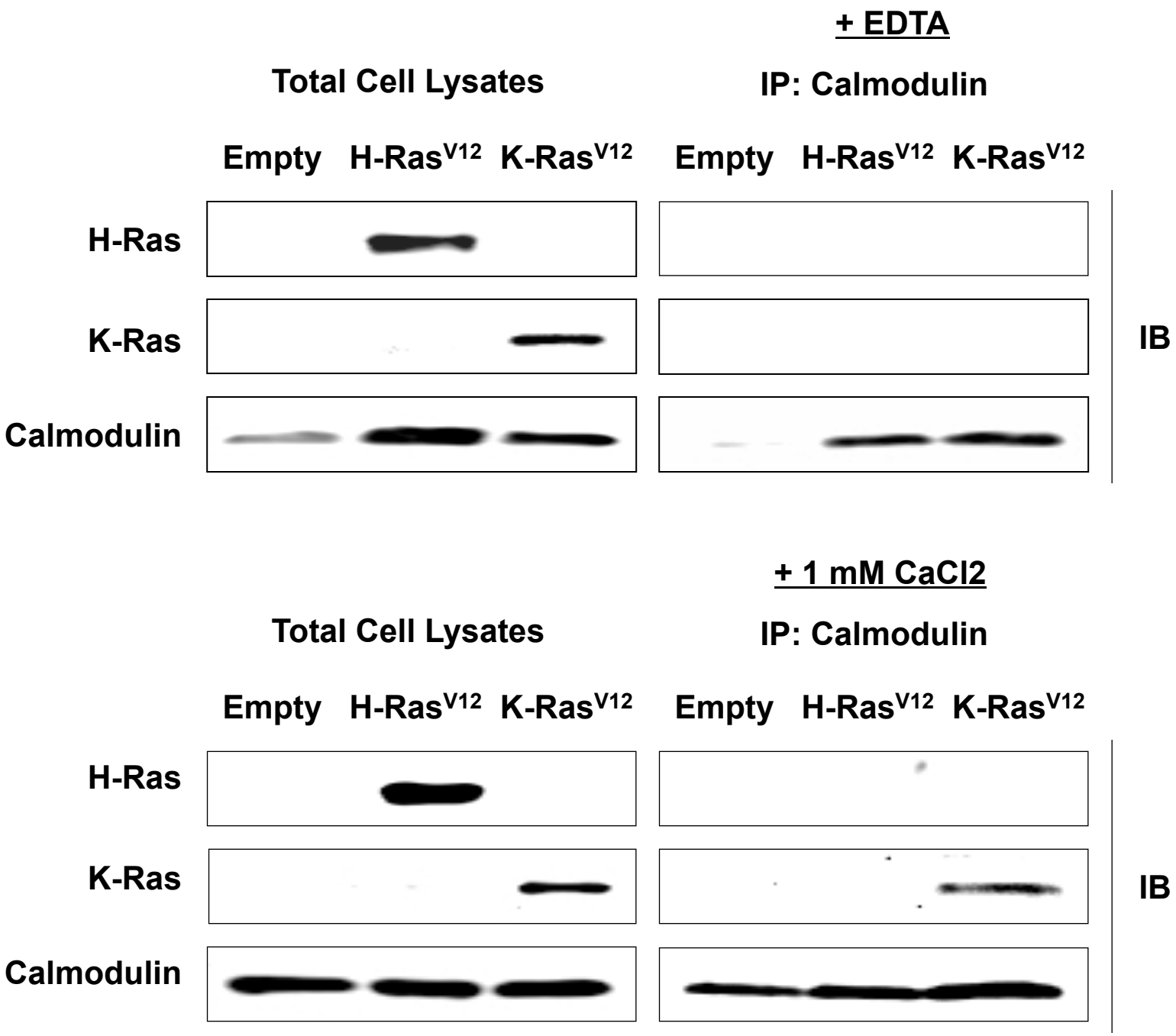
Cont.(5/5) **LIF K.D.(2/5)**



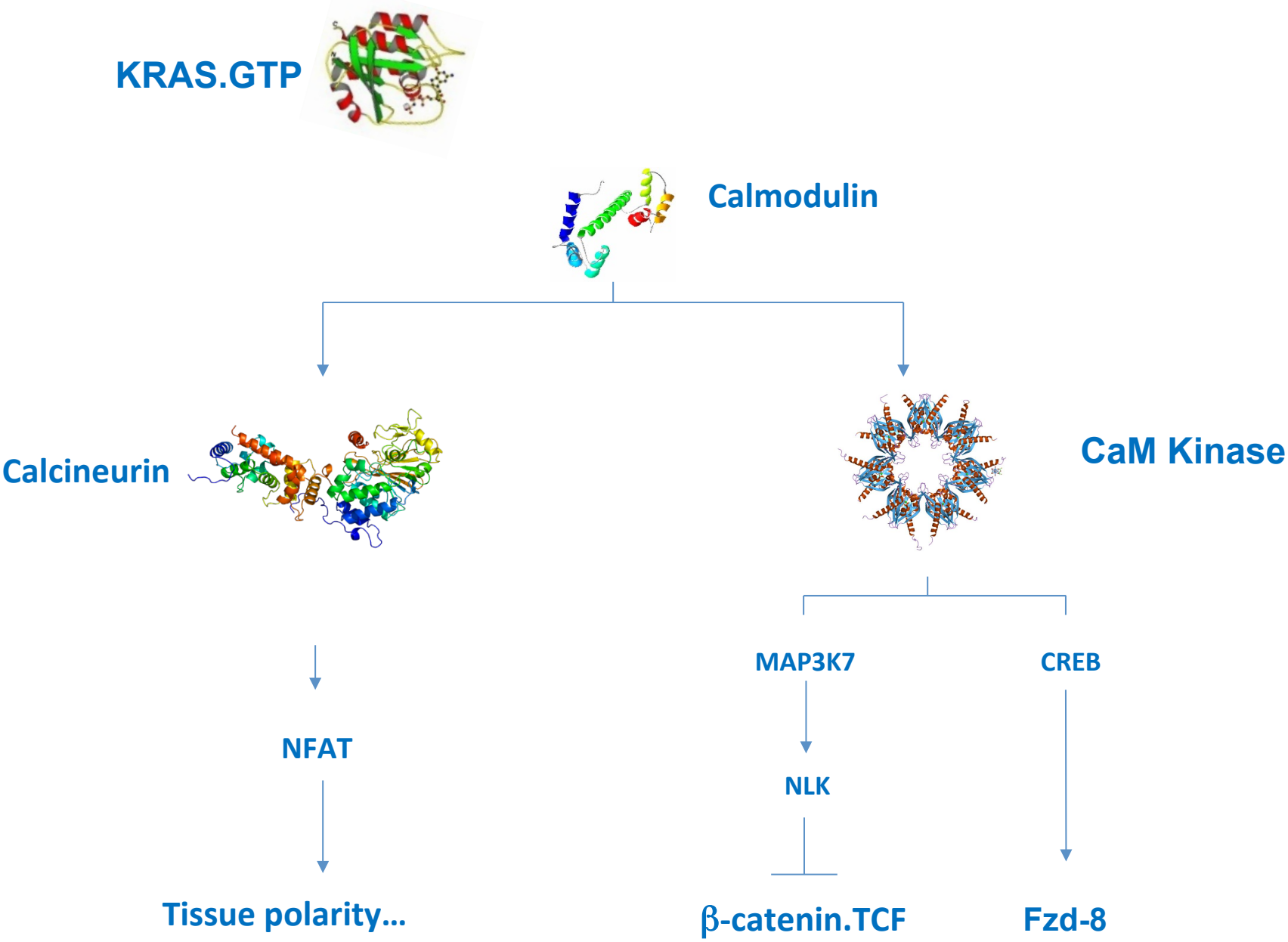
Calmodulin Binds to K-Ras, but Not to H- or N-Ras, and Modulates Its Downstream Signaling

PRIAM VILLALONGA,[1] CRISTINA LÓPEZ-ALCALÁ,[1] MARTA BOSCH,[2] ANTONIO CHILOECHES,[2] NATIVITAT ROCAMORA,[3] JOAN GIL,[4] RICHARD MARAIS,[2] CHRISTOPHER J. MARSHALL,[2] ORIOL BACHS,[1] AND NEUS AGELL[1*]

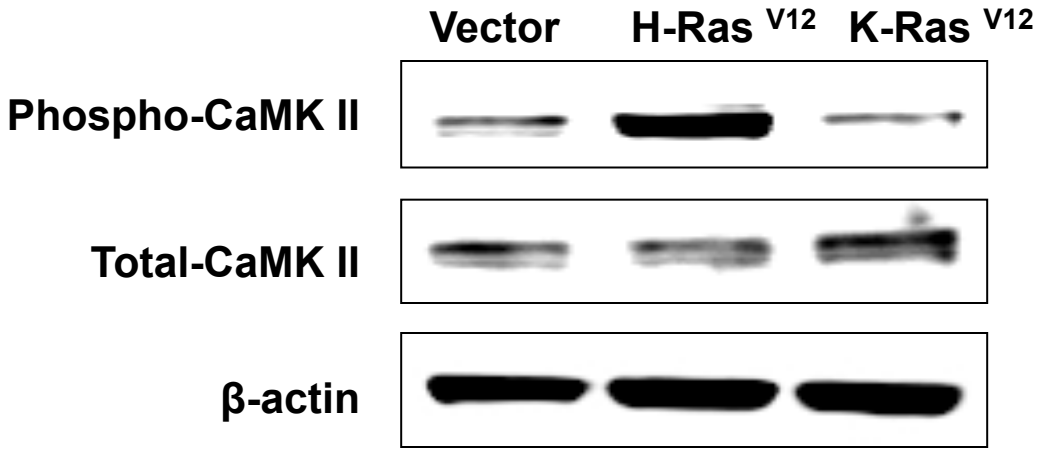


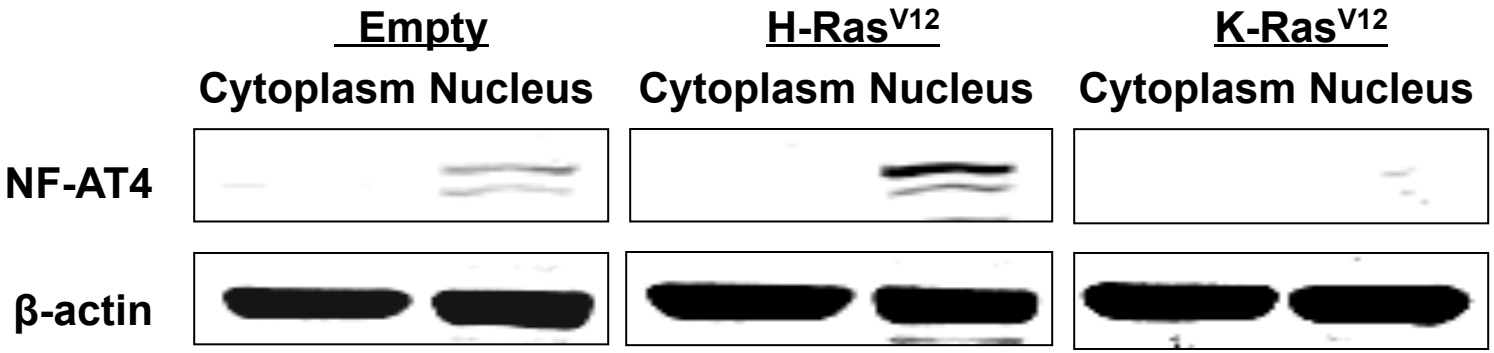

+ EDTA
Total Cell Lysates
IP: Calmodulin
Empty
H-RasV12
K-RasV12
Empty
H-RasV12
K-RasV12
H-Ras
K-Ras
Calmodulin
IB
+ 1 mM CaCl2
Total Cell Lysates
IP: Calmodulin
Empty
H-RasV12
K-RasV12
Empty
H-RasV12
K-RasV12
H-Ras
K-Ras
Calmodulin
IB

KRAS suppresses non-canonical wnt signaling

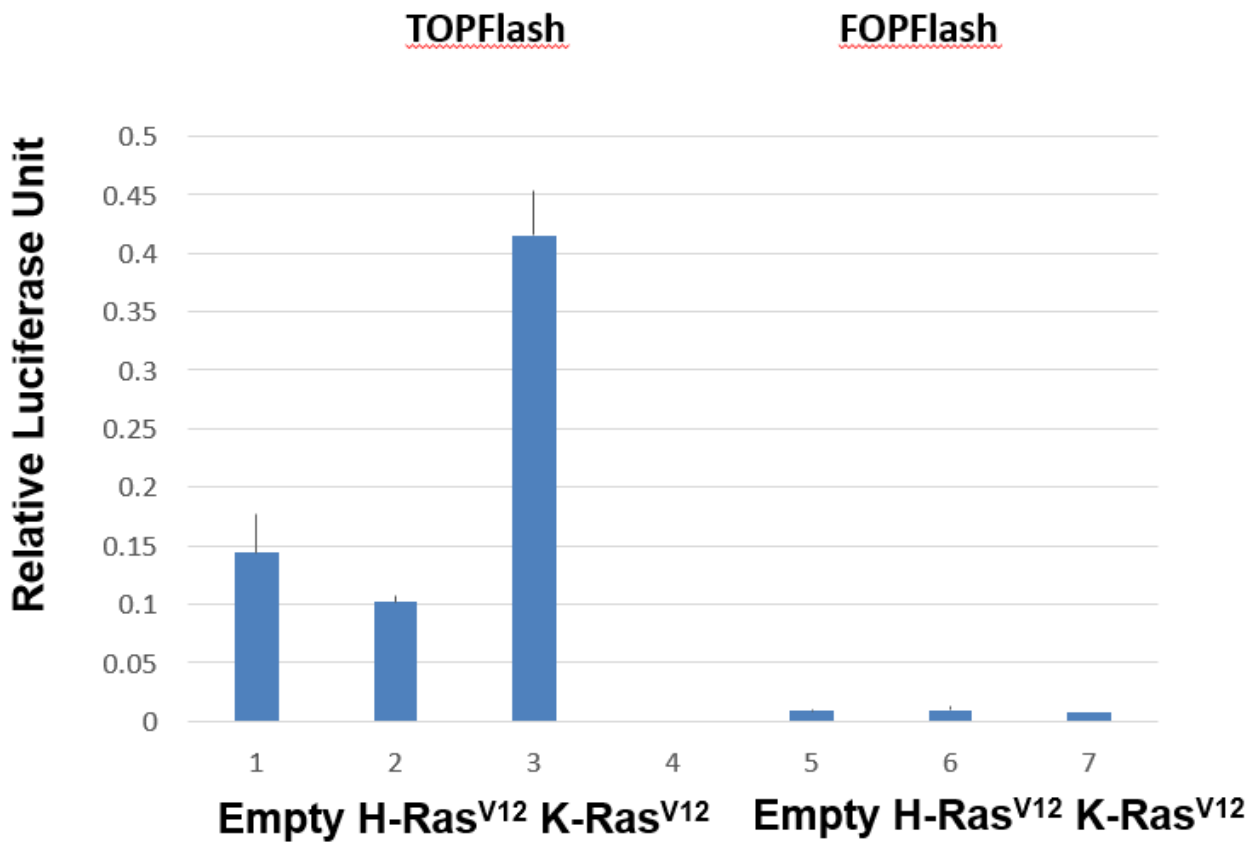


KRAS suppresses non-canonical wnt signaling

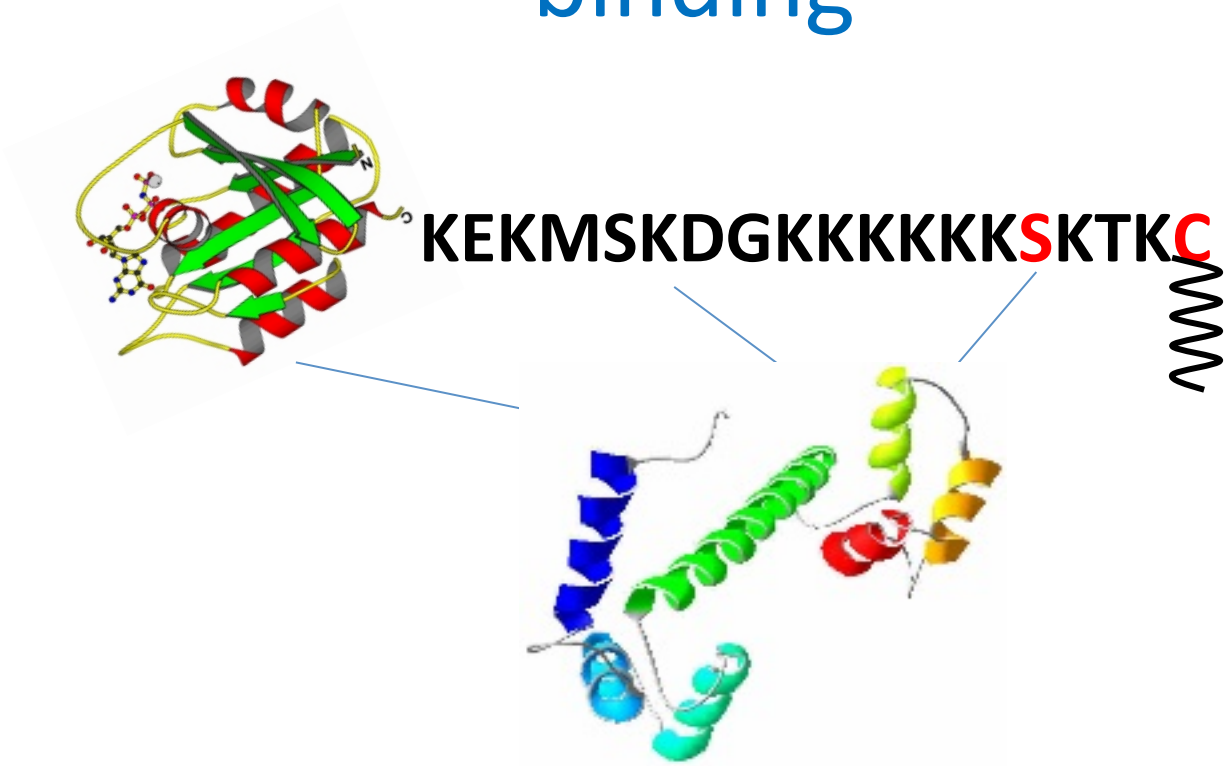


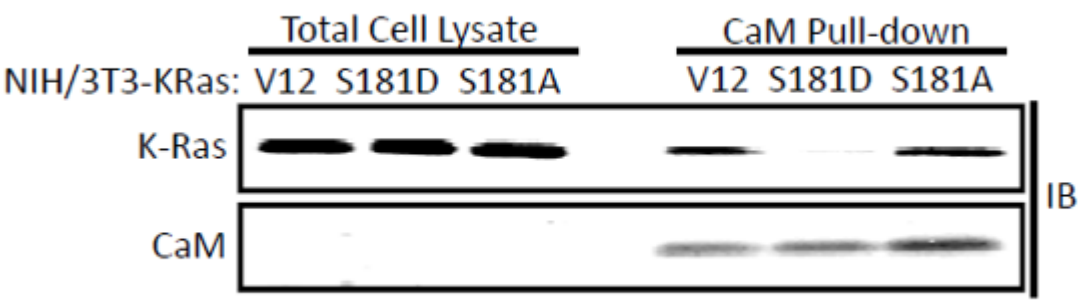


KRAS activates canonical wnt signaling

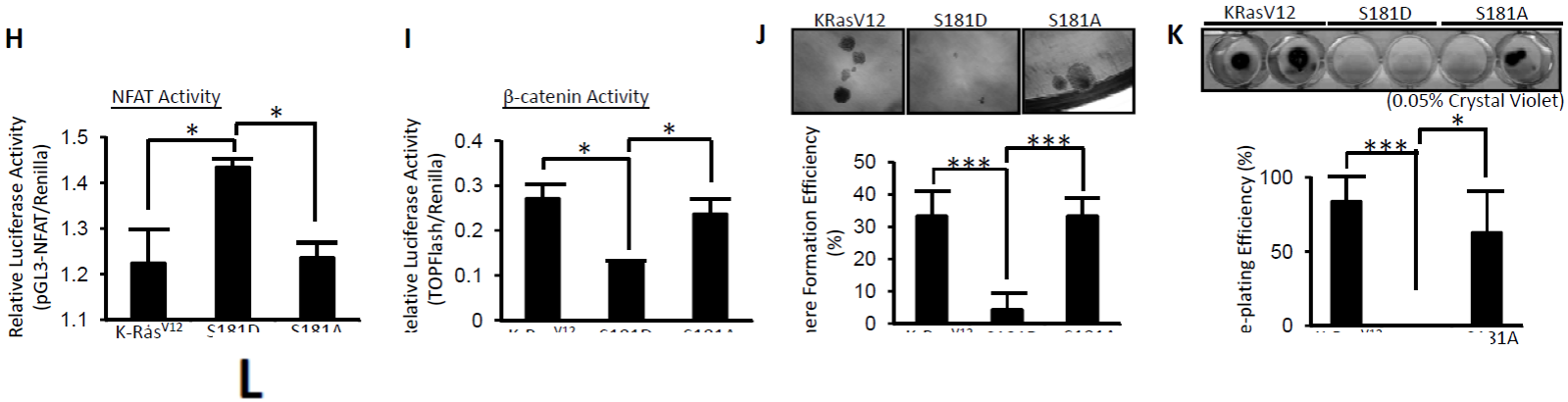


KRAS 181D (or E) prevents calmodulin binding

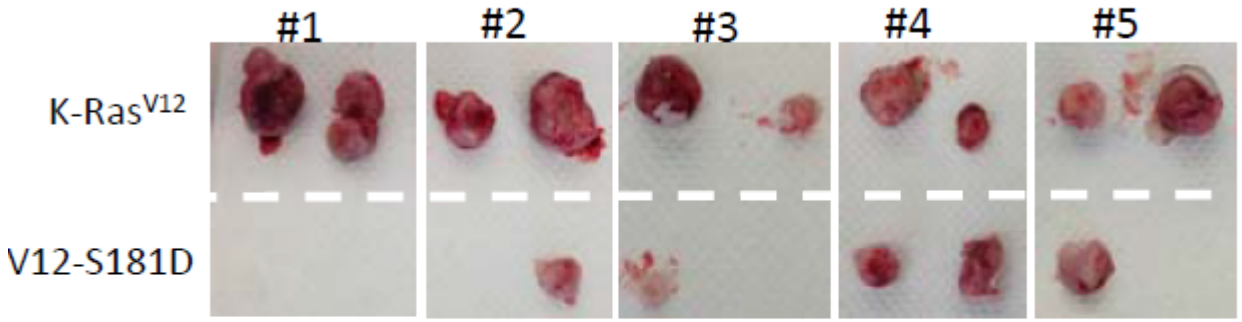




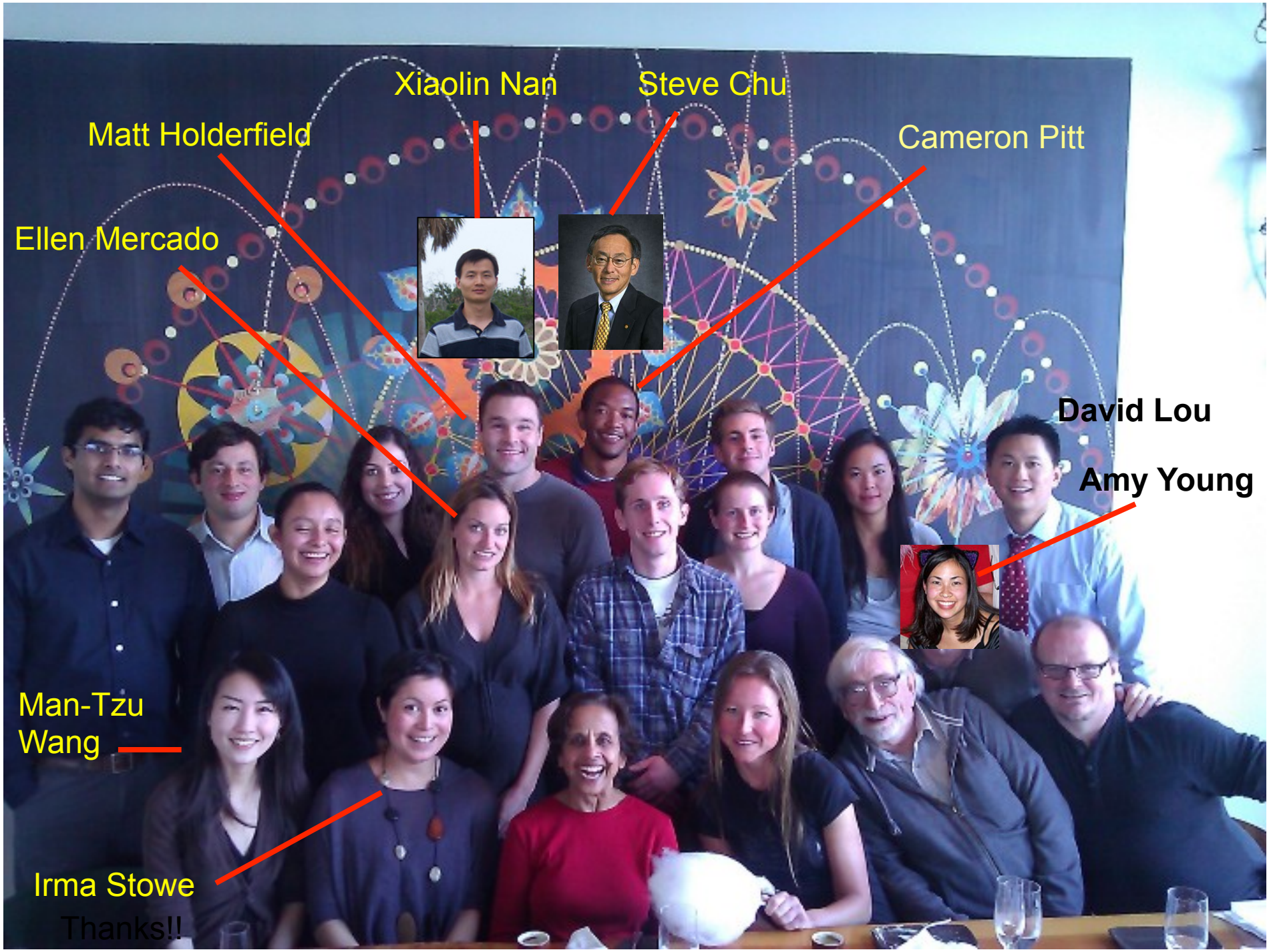
KRAS S181D reverses KRAS stemness



L



Recipients with Developments of Tumors (30th Day)			
	K-Ras^{V12}	S181D	S181A
10^2 cells	10/10	5/10	9/10


Xiaolin Nan
Steve Chu
Matt Holderfield
Cameron Pitt
Ellen Mercado
David Lou
Amy Young
Man-Tzu Wang
Irma Stowe
Thanks!!