

Frederick National Laboratory for Cancer Research



Ras Project Progress Report

Frank McCormick

Ras Mutations in Cancer



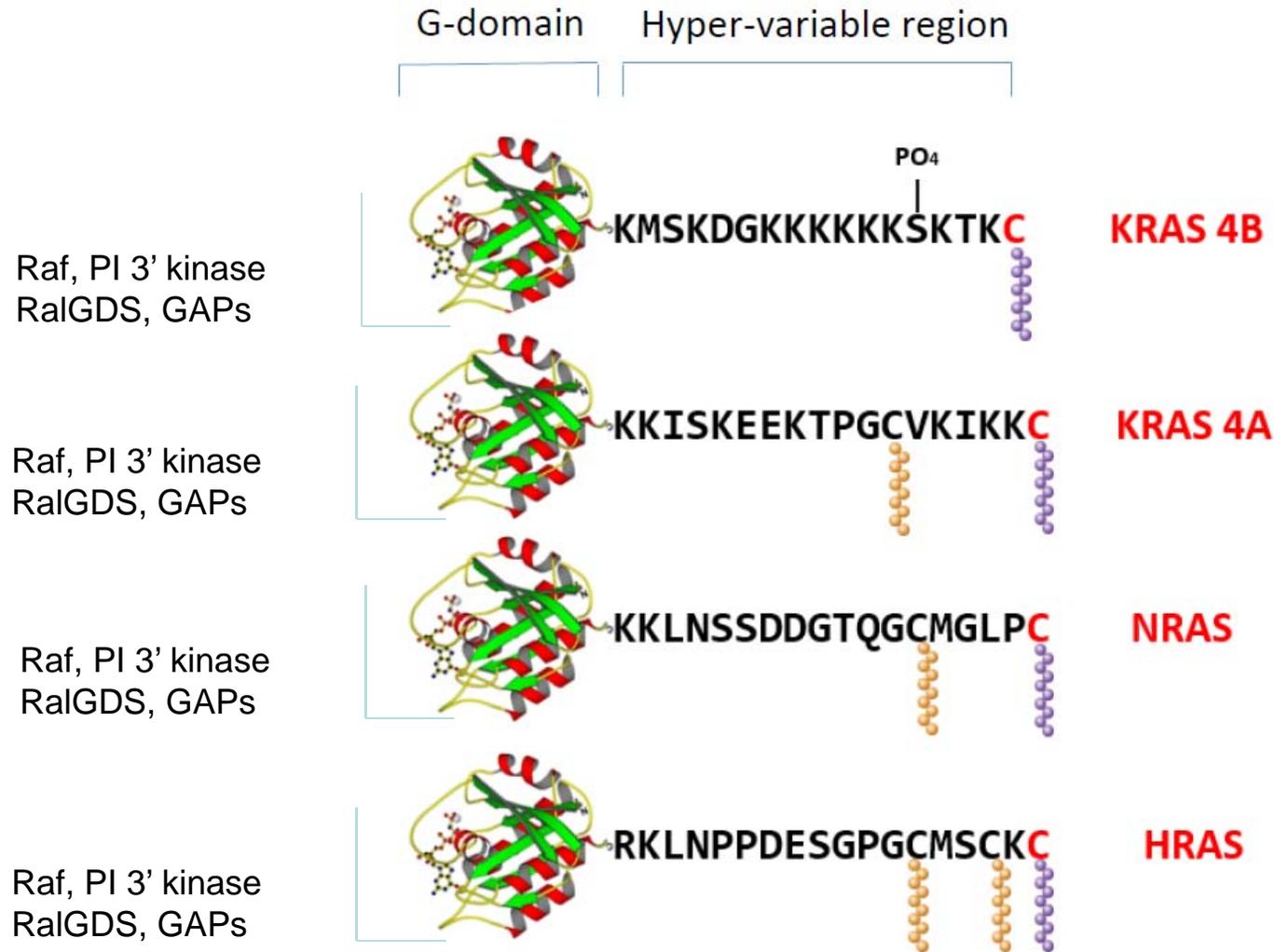
	KRAS	HRAS	NRAS	Total
Primary Tissue	%	%	%	%
Pancreas	71	0	<1	71
Colon	35	1	6	42
Small Intestine	35	0	<1	35
Biliary Tract	26	0	2	28
Endometrium	17	<1	5	22
Lung	19	<1	1	20
Skin	1	1	18	20
Cervix	8	9	2	19
Urinary Tract	5	10	1	16

K-Ras Mutations in Four Major Cancers

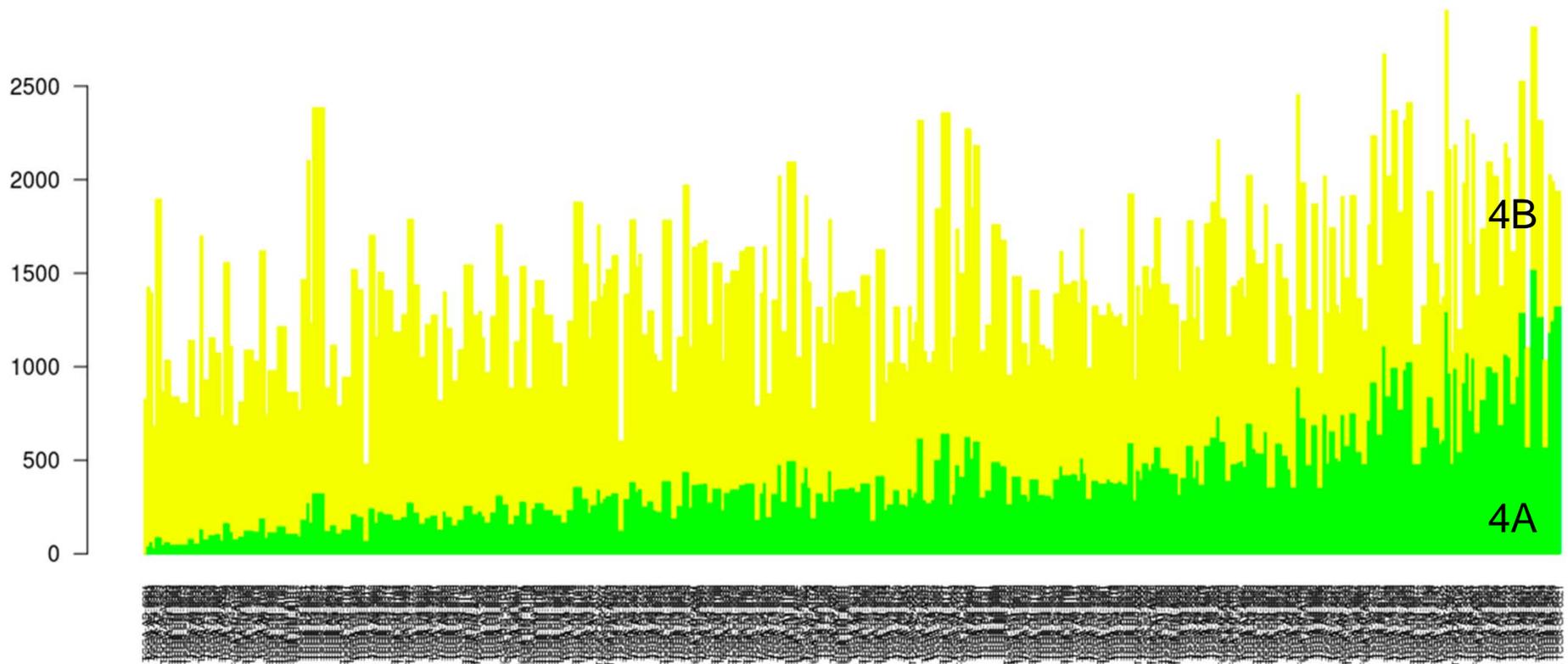


	All KRAS	G12C	G12D	G12V	G13D
Colorectal	60,000	5,700	25,000	15,700	13,600
Lung	45,600	23,000	9,200	11,900	1,500
Pancreas	32,200	1,000	19,500	11,500	200
Total new cases/yr	137,800	29,700	53,700	39,100	15,300

Ras Proteins

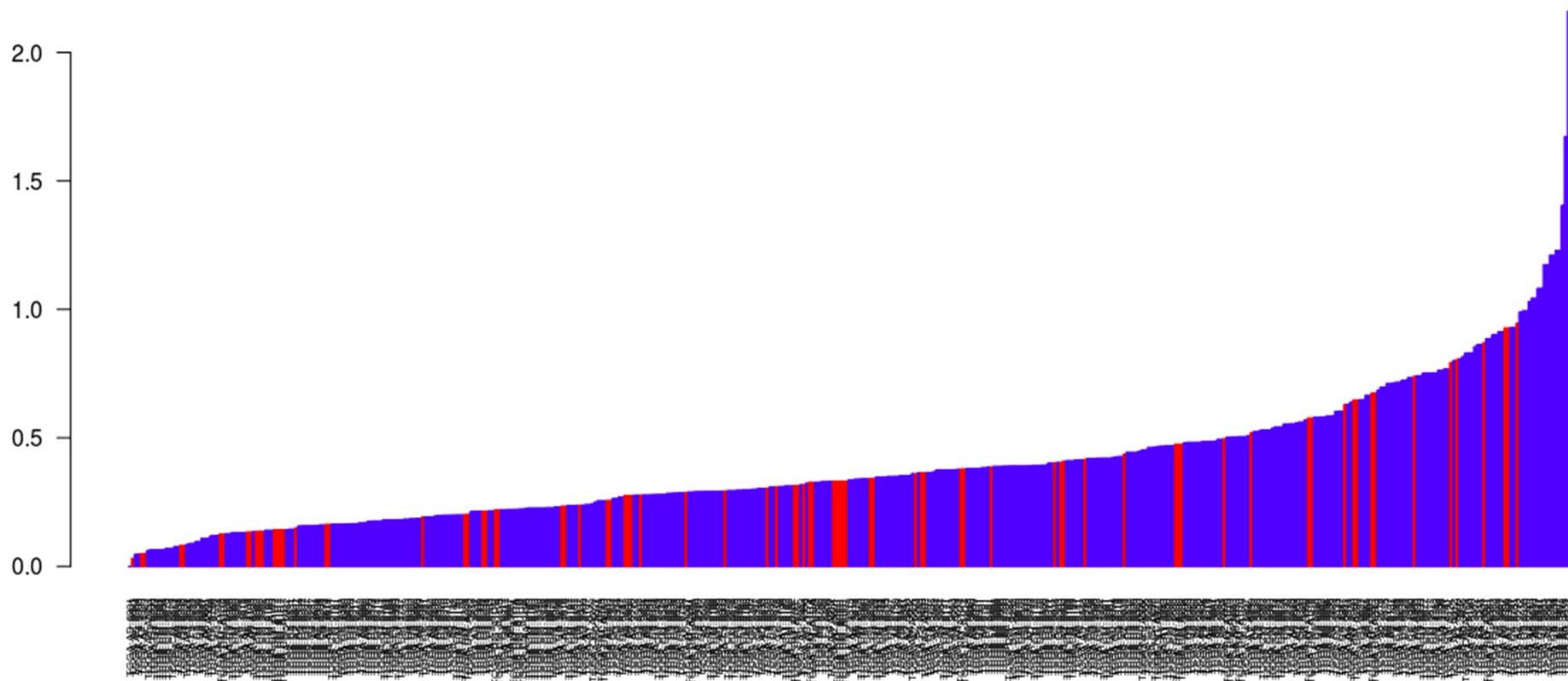


Expression of KRAS 4A & 4B in Colorectal Cancers



Bob Stephens

Colorectal Cancers by 4A/4B Ratio (TCGA)



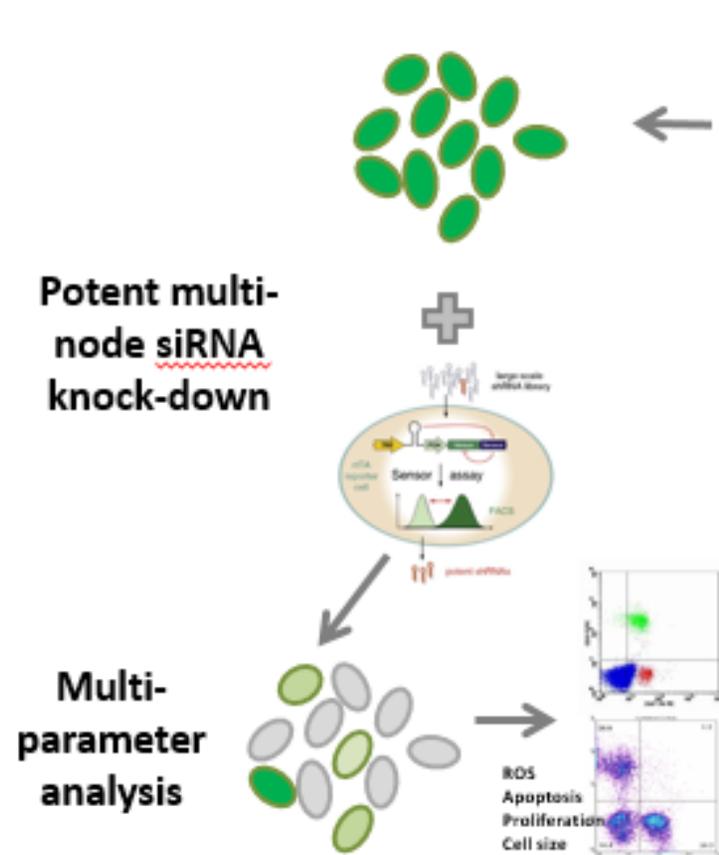
Blue=alive; red=dead

Project Zero: Validating KRAS, Identifying KRAS dependent cells

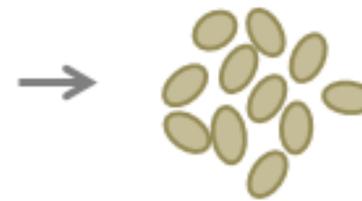


Validate KRAS and Downstream Signaling Nodes Pancreatic Cell Lines

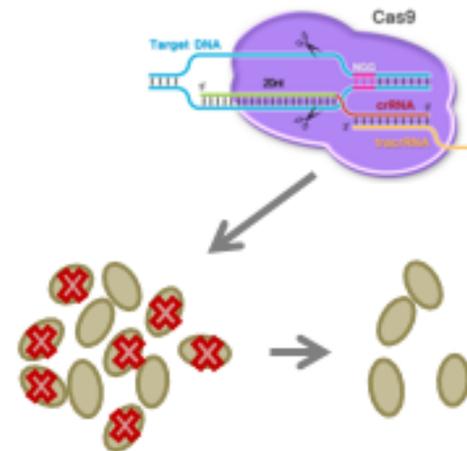
Mechanisms of Resistance



Cell line intake
QC, Characterization



Ablate KRAS using CRISPR



Characterize resistant cells

Project Zero: Progress



Assays

- Cell-based assays standardized
 - Viability/proliferation, apoptosis, anchorage independence (agar vs polyhema), migration/invasion

Cell Lines

- >30 cell mutant KRAS cell lines fully characterized
 - Growth, GE, protein expression, anchorage independence, migration/invasion

Reagents

- Validation of KD/KO reagents ongoing
 - CRISPR, inducible lentivirus
- Novel cell lines developed (P2, P3), validation ongoing

Project Zero: Timelines



1. Validate KRAS and downstream signaling nodes

- Collaboration with Tina Yuan, UCSF and Cyril Benes (Harvard, MGH) has been initiated
 - Reagent, cell lines and protocol transfer ongoing
- Pancreatic cell line characterization using this method expected to be complete within 4-5 months

2. Mechanisms of resistance

- Validating CRISPR method
- Cell line panel is being generated using characterization data
- Full method will be on-line within 2-3 months

Informatics approach to KRAS Dependency



- Cell line selection for the different RAS program projects benefits from full genomics assessment of the genetic background of the cells.
- Previous reports have identified panels of KRAS-dependency genes associated with various tumorigenic indicators such as EMT
- This information can be combined with our project derived data to aid in interpretation of results (responder/non-responder etc.)

Cancer Cell. 2009 Jun 2;15(6):489-500. doi: 10.1016/j.ccr.2009.03.022.

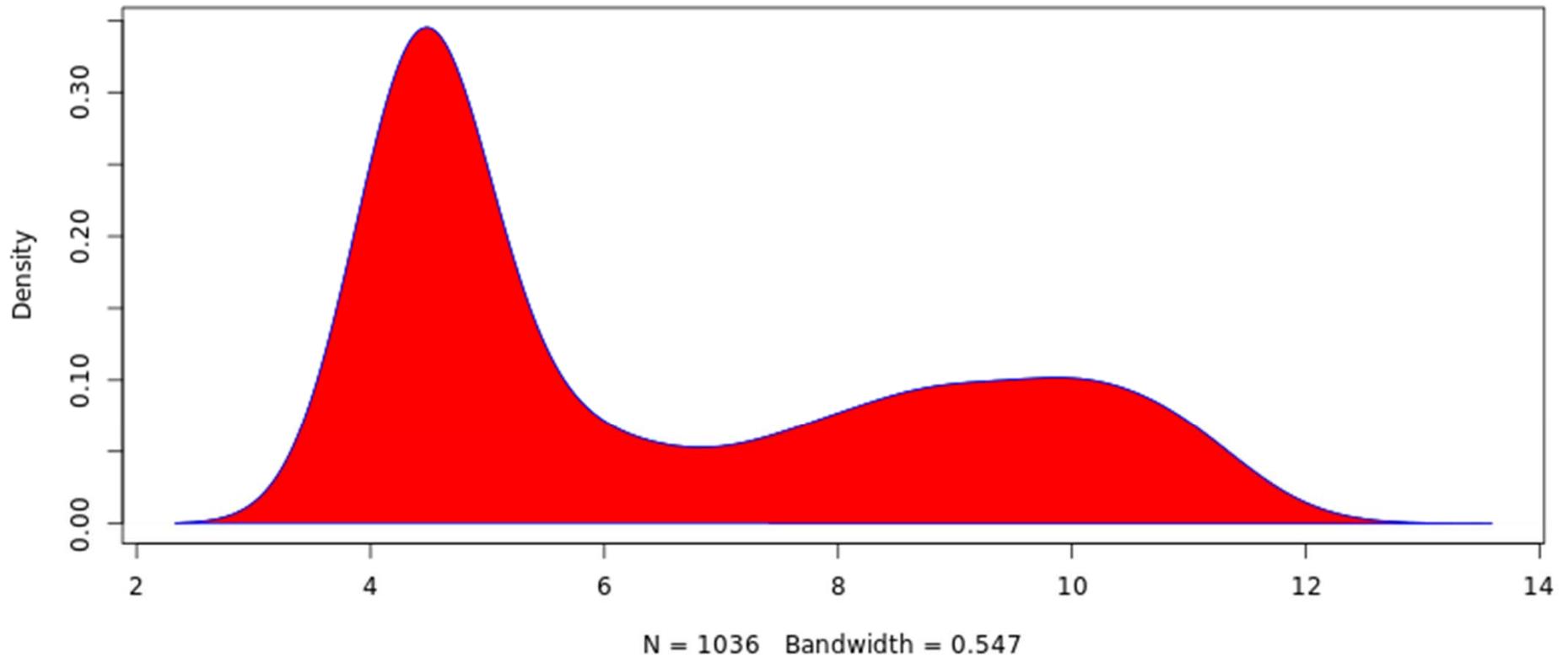
A gene expression signature associated with "K-Ras addiction" reveals regulators of EMT and tumor cell survival.

[Singh A, Greninger P, Rhodes D, Koopman L, Violette S, Bardeesy N, Settleman J.](#)

Cadherin expression across all ccle cell lines



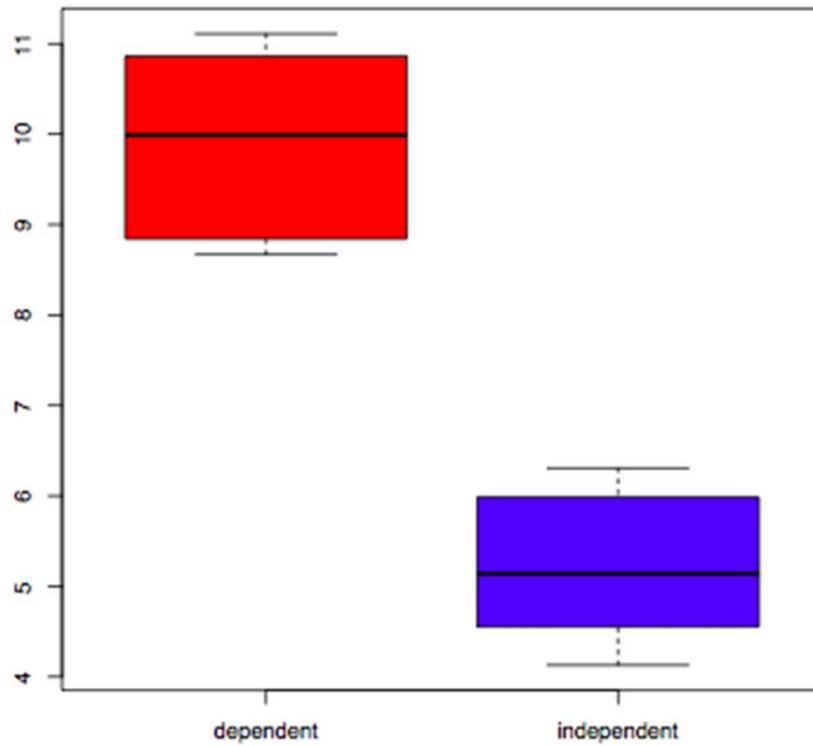
Density plot for `cdh1/expr_bygene/score`



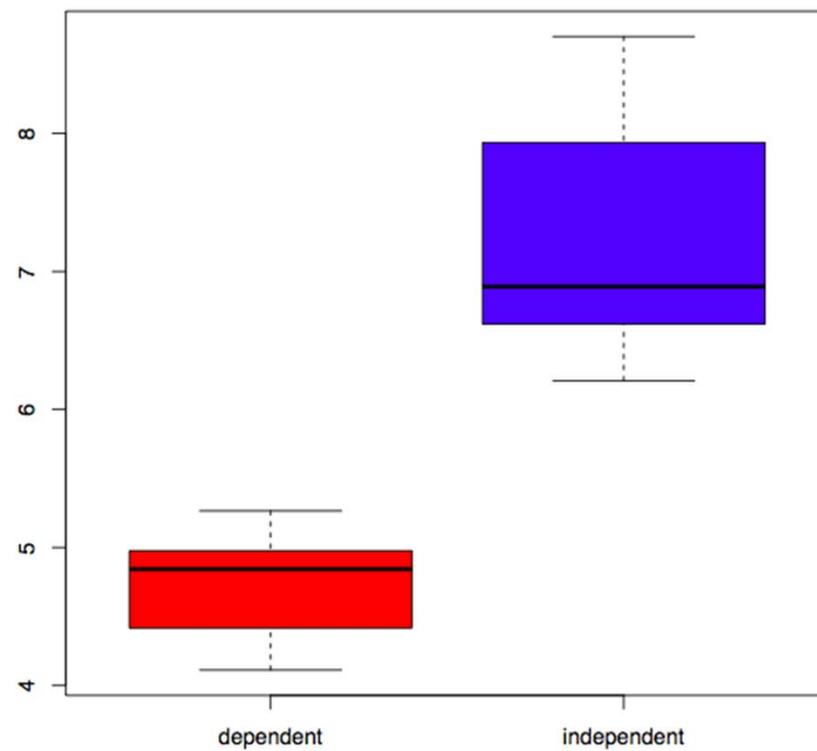
K-Ras dependent cells and EMT



Plot for CDH1 lengths=8,8



Plot for ZEB1 lengths=8,8



Project One: Overall goals



- Identify new pockets for drug binding, potential for affecting GTP hydrolysis, allosteric regulators, binding assays
- Structural and biophysical analysis of 4 KRAS mutants bound to key effectors and regulators (Raf, PIK, RalGDS and GAP)
- Structure of KRAS4B bound to calmodulin
- Structure of full length Raf bound to Ras
- Processed Ras bound to synthetic membrane

Project One: KRAS Structural and Biophysical Analysis



- Structural/biochemical analysis of KRAS mutants.
 - Pilot crystallization for KRAS variants (WT KRAS, G12D, G12V, G12C, and G13D)
 - Analysis of GTPase activity, GDP release, effector binding (IP or SPR)
- Analysis of the KRAS-Calmodulin interactions.
 - Crystals of KRAS-Calmodulin complex
 - Analysis of the binding determinants/affinity using SPR, IP, fluorescence polarization
 - Effect of oncogenic mutations/GTP-GDP state on the binding interaction

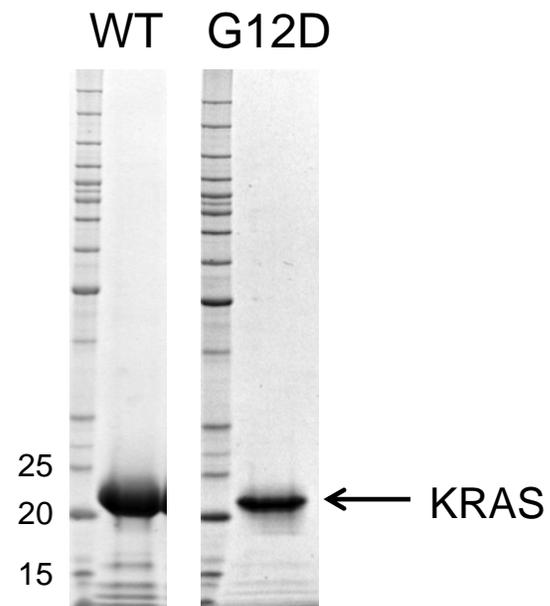
Protein Production



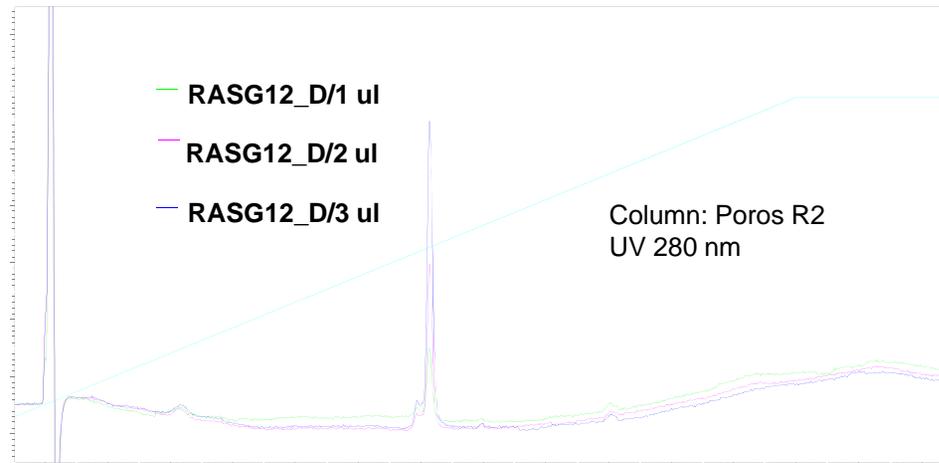
- Expressed in *E. coli* and insect cells as His6-tev-, and His6-MBP-tev-
 - New constructs screened by micro-scale purification
 - Scale up to 2 or 15 liter scale
- Purification process: IMAC/TEV digestion/IMAC/SEC
- Yields from His6-MBP- constructs (~100+ mg/liter) are 10-fold better than His6- constructs
- Optimization of insect expression ongoing

Protein purified from 2 liters *E. coli* culture

	His6-MBP-	
	His6-tev	tev-
WT	13.5	135
G12D	20	133

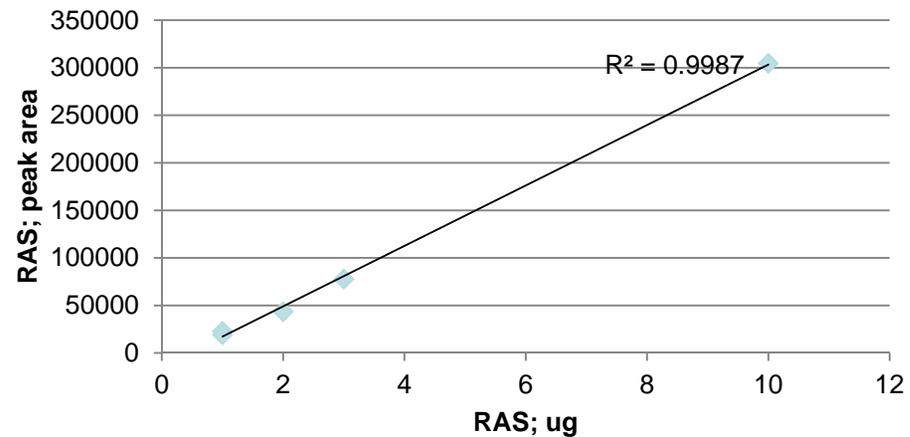


Quantitative Analysis of KRAS by Amino Acid Analysis and HPLC

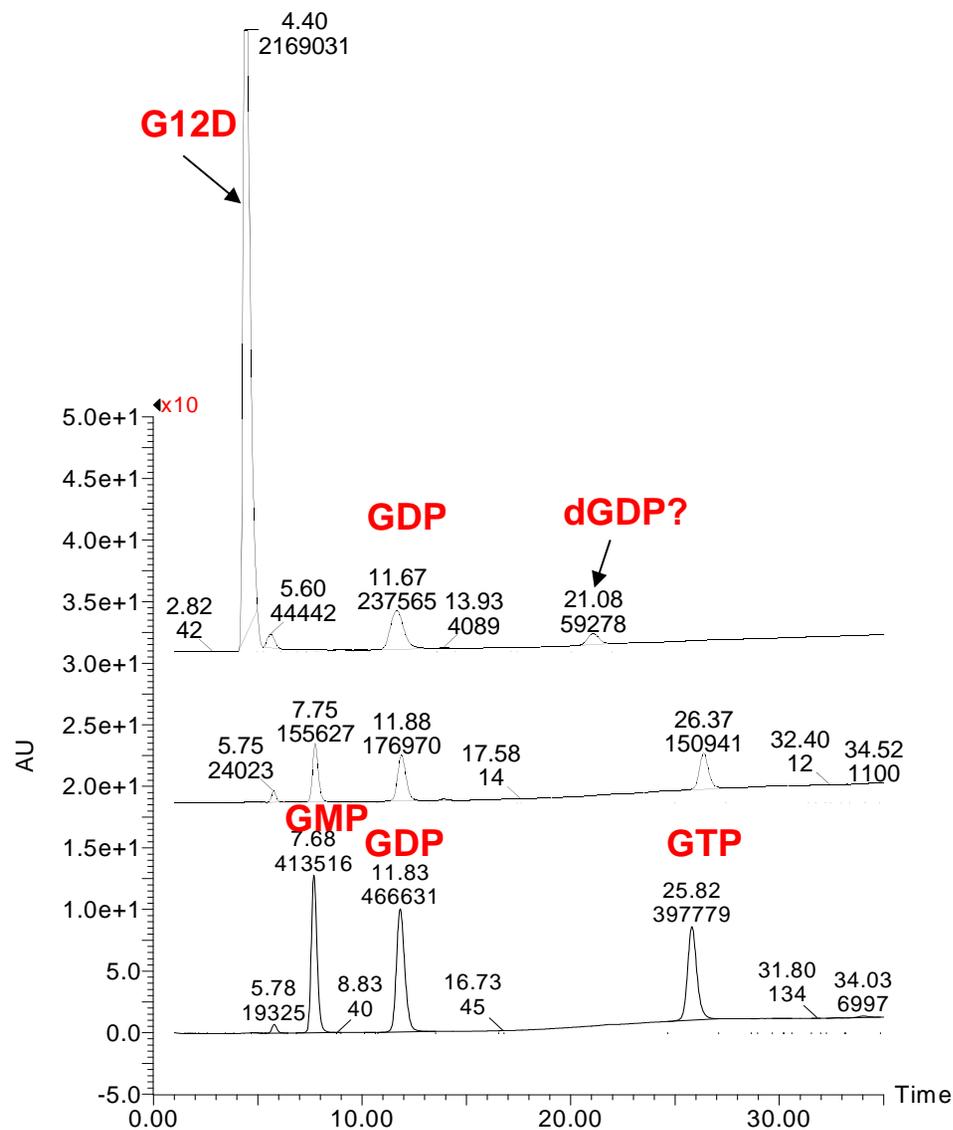


RAS calibration by HPLC; 280 nm

	RAS; ul	280 nm	RAS; ug (aaa)
	1	22634	1.265
	1	19222	1.265
	2	43394	2.53
	3	77617	3.795
	10	304657	12.65
ras,G12D-GTP	20	11280	1.034
ras, G12D	30	12264	1.073



Determination of GXP Concentration in G12D RAS Prep



RAS GDP loading =
26%
(no added GDP)

10 μM, G12D prep

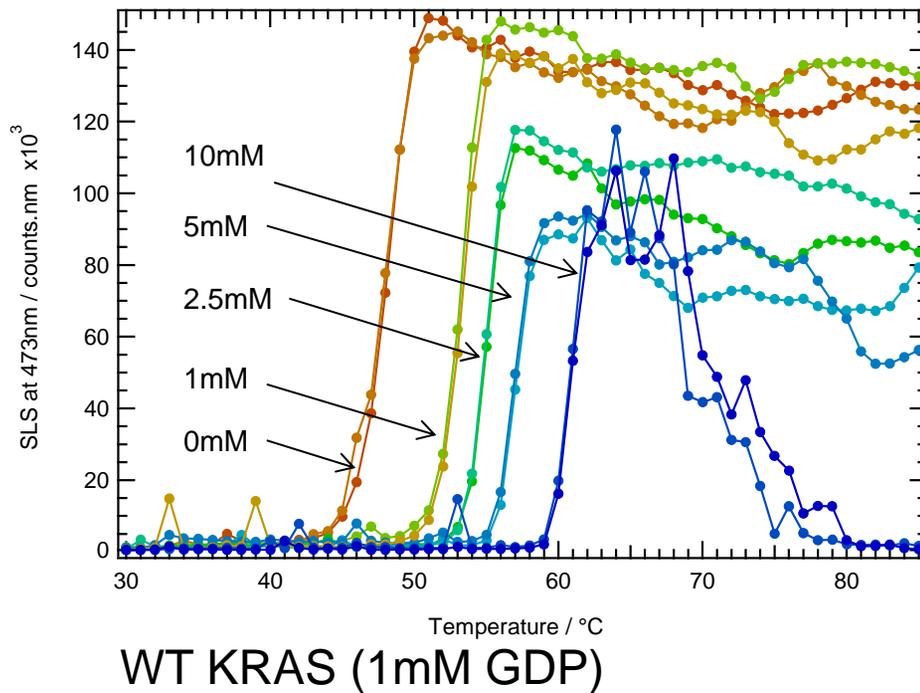
2 μM, GXP mix

5 μM, GXP mix

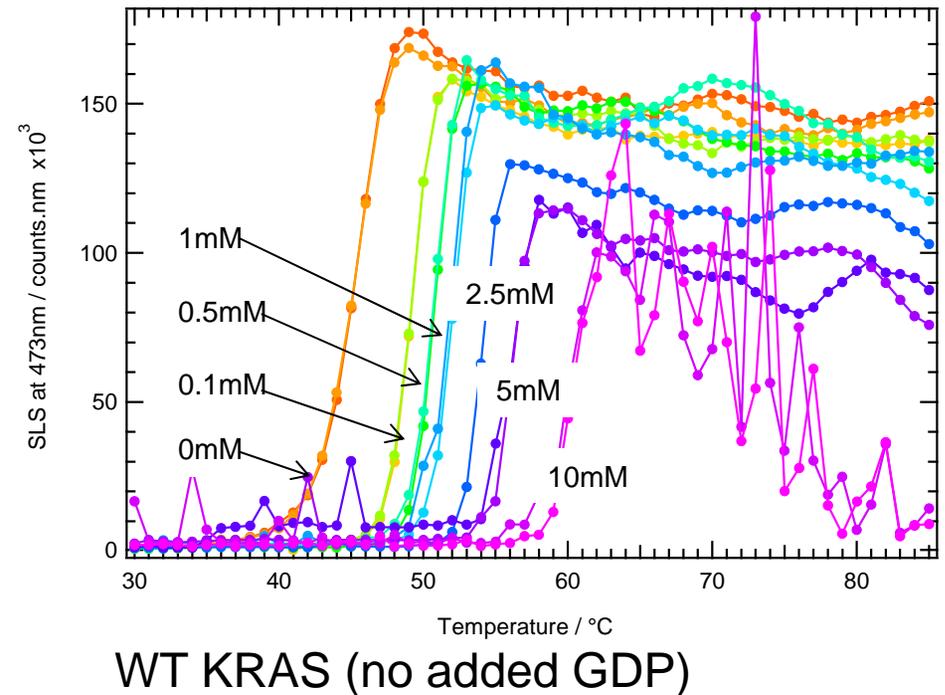
Addition of GDP increases the Stability of WT KRAS



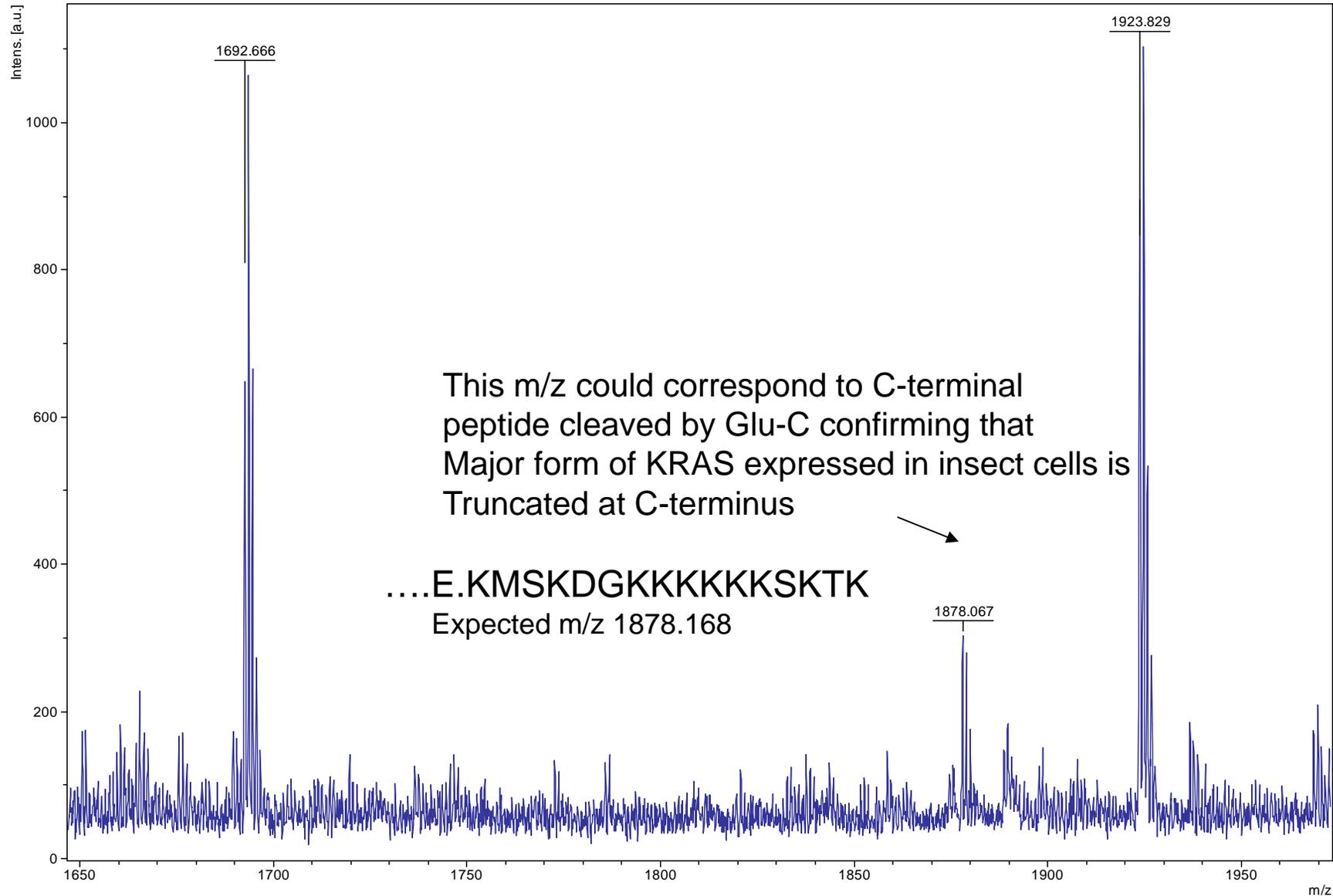
Static light scattering 473nm



Static light scattering 473nm



MALDI-TOF MS of KRAS 4B (wt) Expressed in Insect Cells

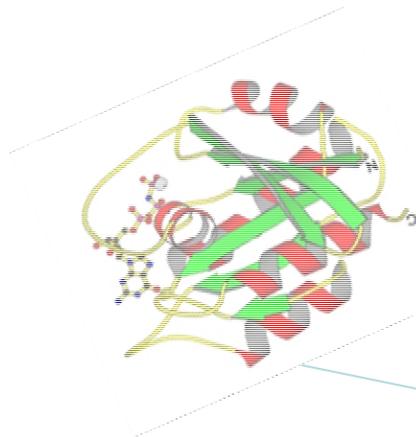


KRAS : Calmodulin Interaction



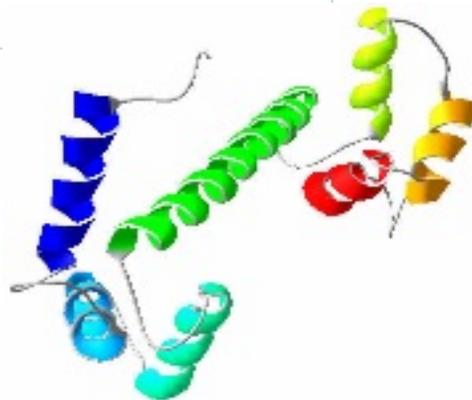
Calmodulin Binds to K-Ras, but Not to H- or N-Ras, and Modulates Its Downstream Signaling

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*}

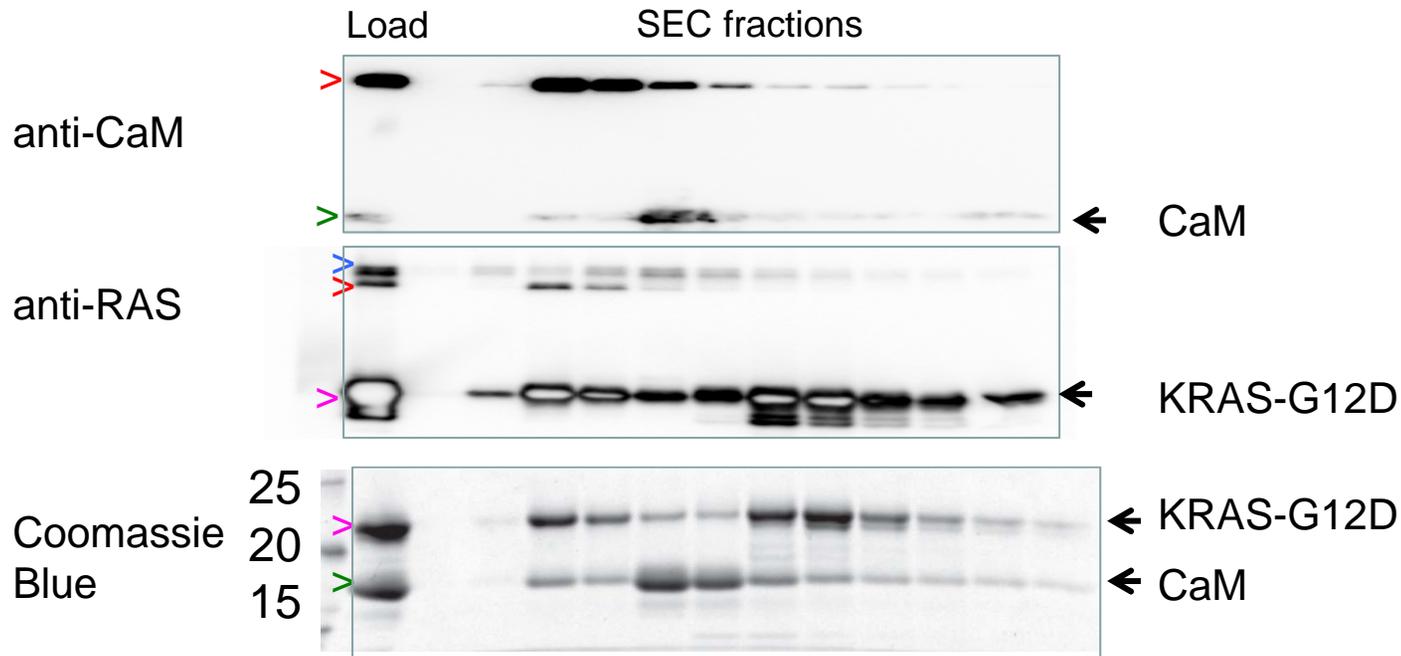
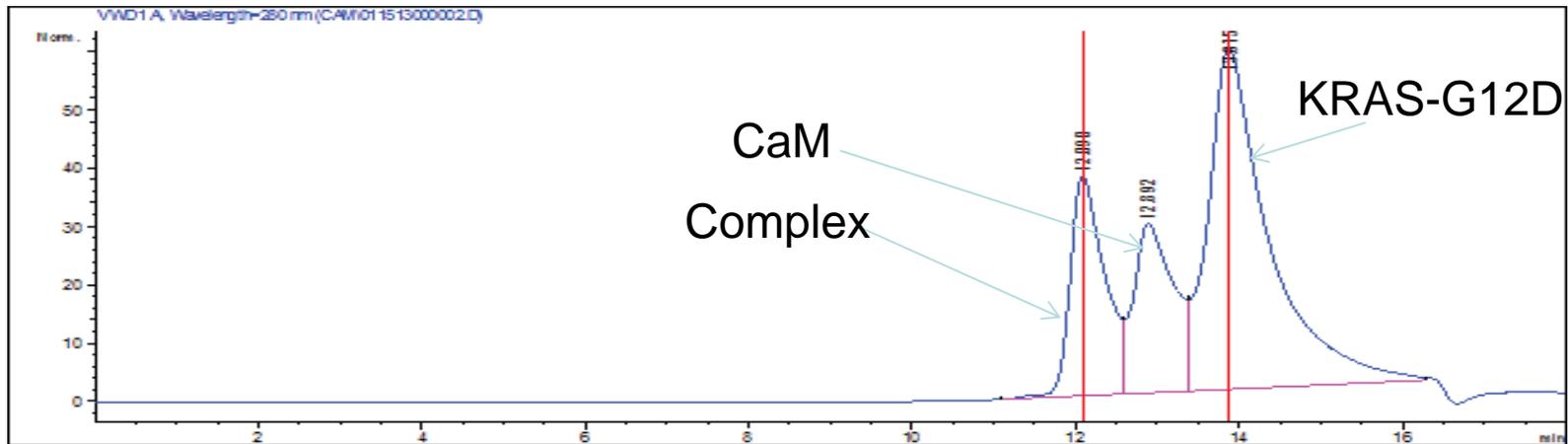


KRAS 4B

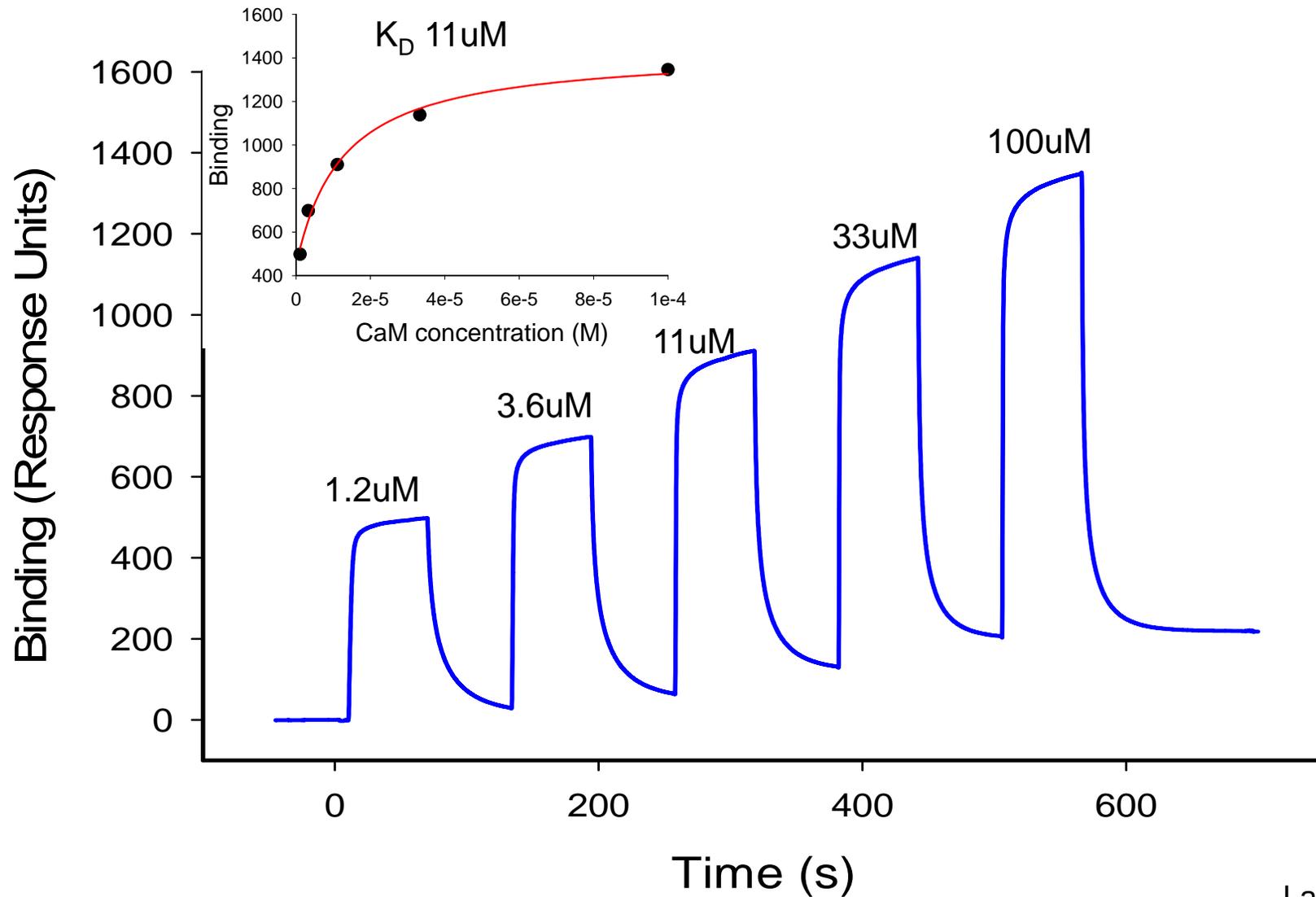
KEKMSKDGKKKKKKSKTKC



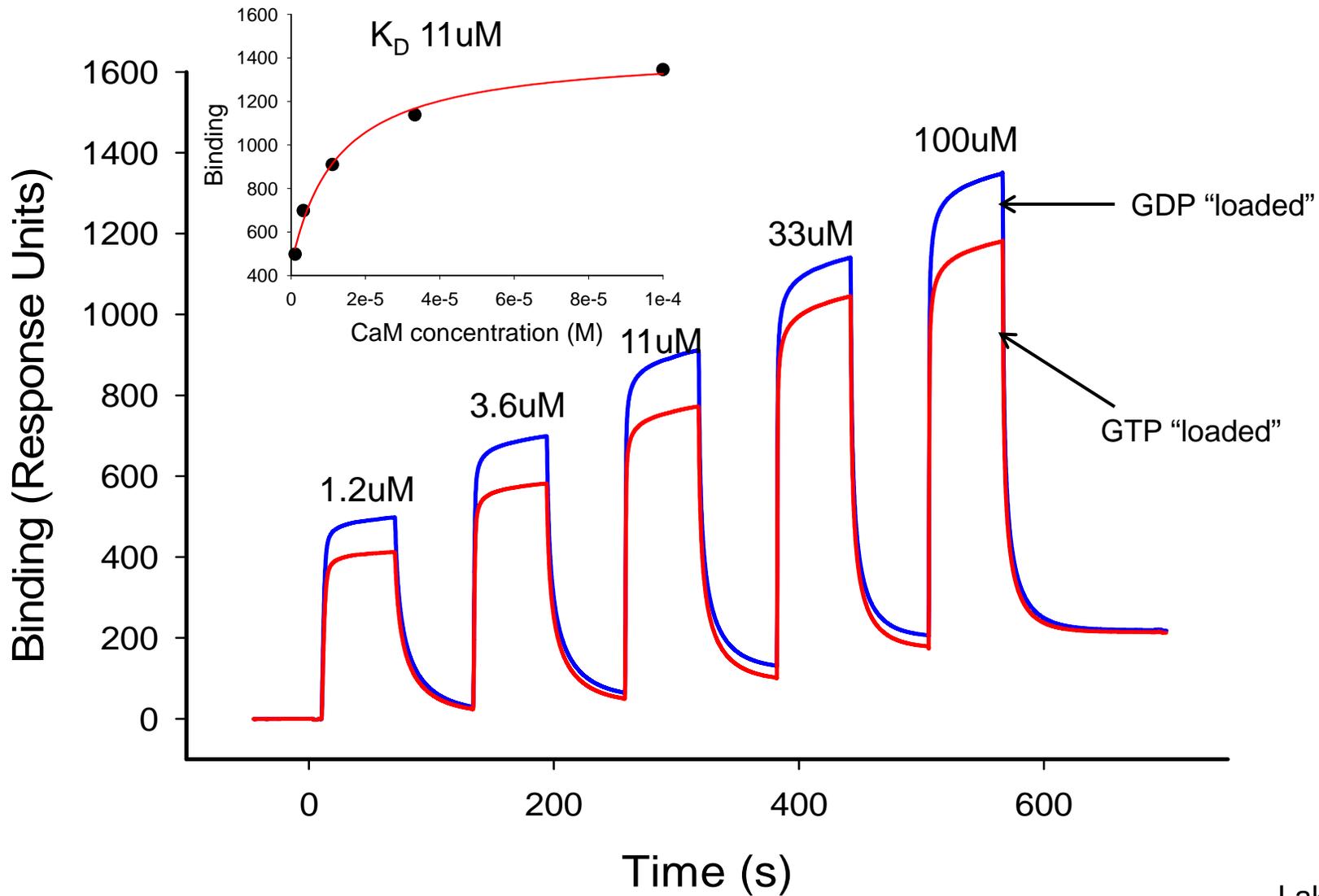
KRAS-G12D-CaM Complexes by SEC and Western Blot



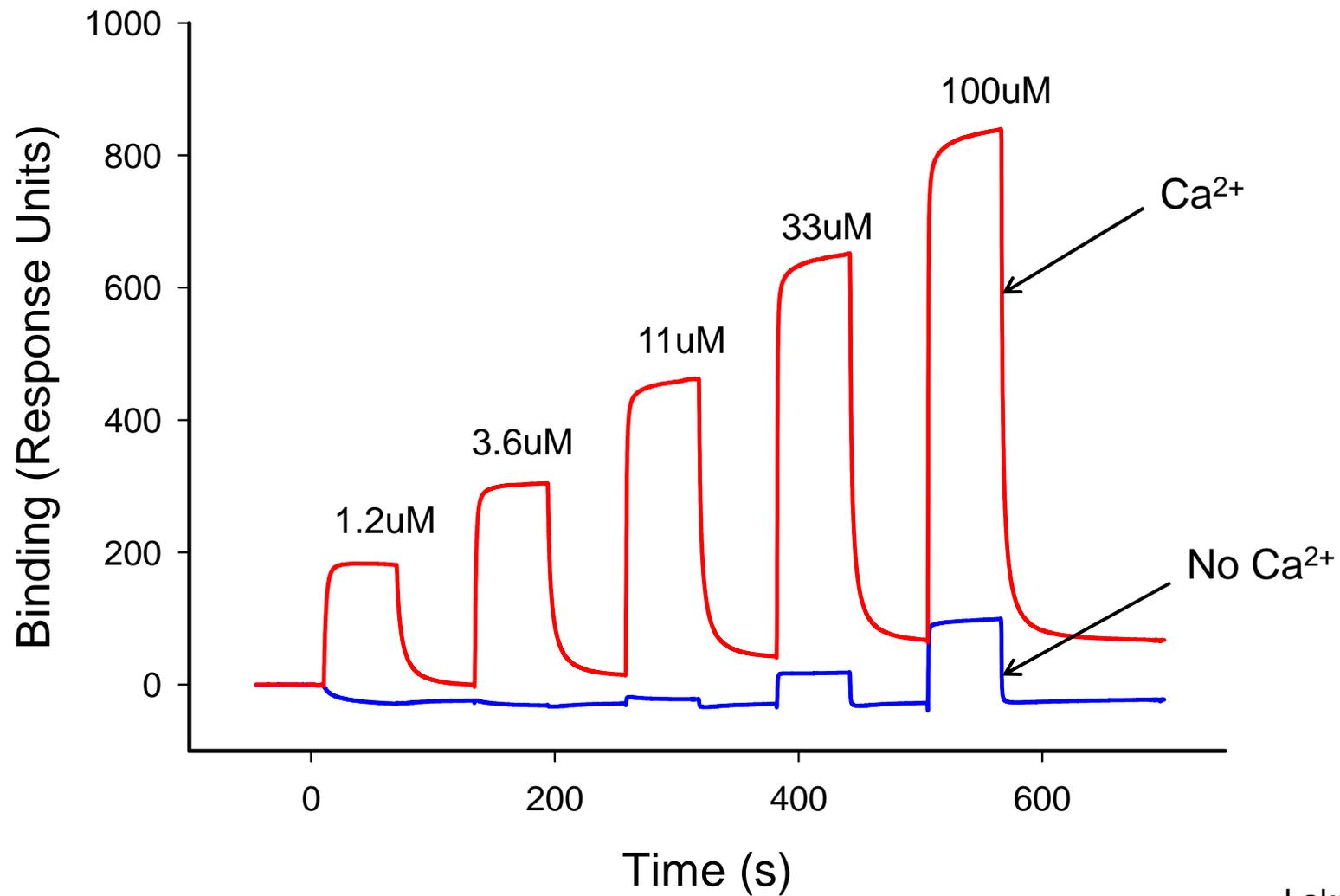
Binding kinetics of CaM to WT-KRAS



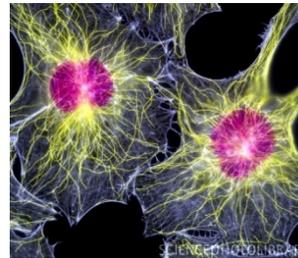
Binding Kinetics of CaM to WT-KRAS



CaM binding is Ca^{2+} Dependent



Project Two: Cell-based Screens for Compounds that Target K-Ras



RAS-less MEFs

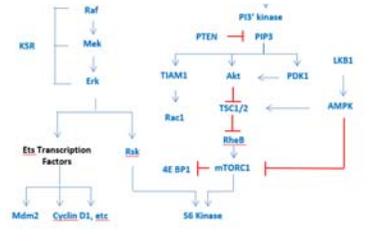
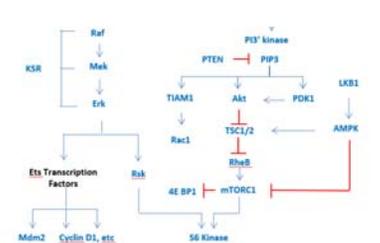
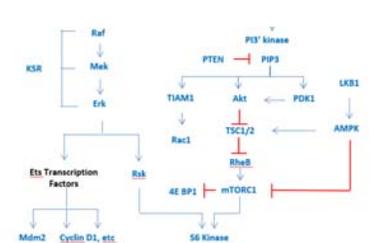
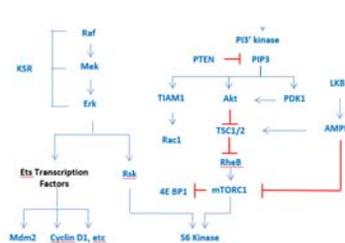
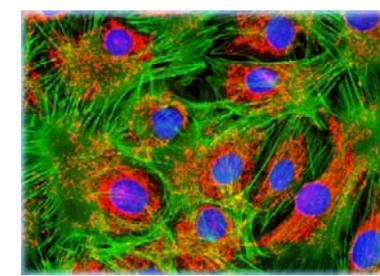
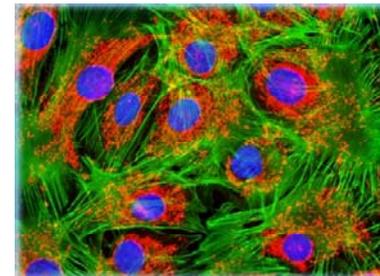
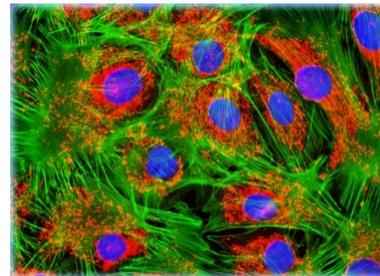
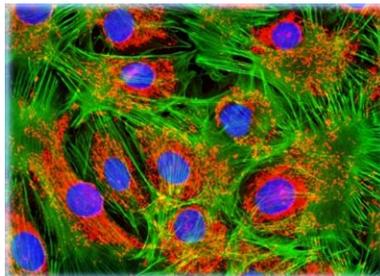
Drosten M, Dhawahir A, Sum EY, Urosevic J, Lechuga CG, Esteban LM, Castellano E, Guerra C, Santos E, **Barbacid M**. EMBO J. 2010

HRAS

NRAS

KRAS4A

KRAS4B



Work with NCATS to Develop 4A/4B Screen



- Presentation of RAS Program to NCATS Dec 19, 2013
- Developed 4A/4B screen strategy and workplan; presented to NCATS on January 17, 2014
- Discussions lead to strategy that meets RAS Program goal to find hits that target unique KRAS features
 - 1st identify compounds that differentially effect mutant KRAS 4b relative to wt HRAS
 - then focus on the difference between KRAS 4a and KRAS 4b hypothesis is that the unique *KRAS* carboxy-terminus contributes to *KRAS* dependent oncogenesis

Iterative Assay Development

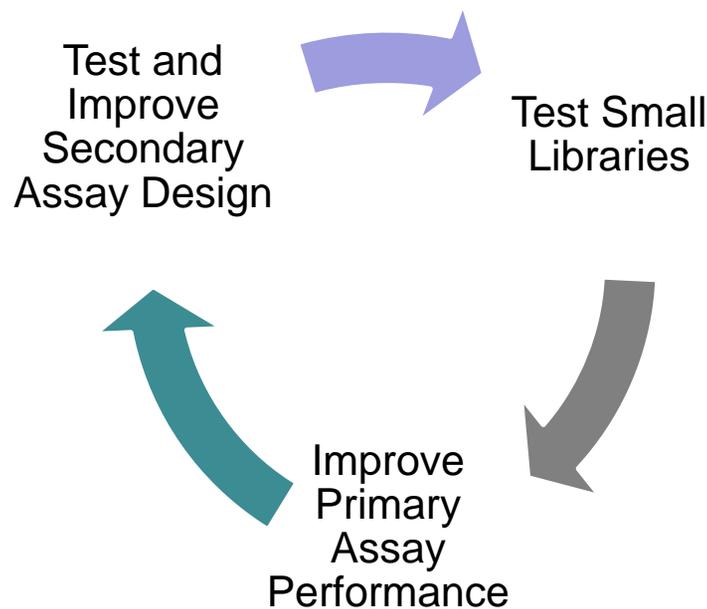


- Proximity of NCATS to ATRF imparts advantages to assay optimization and qualification
- Division of Labor:
 - Ras Program
 - Develop cell lines
 - Proof of principle two color growth readout
 - Confirm response to FTIs
 - Develop secondary screens
 - Cellular, biophysical, high-content imaging
 - NCATS:
 - Provide advice and expertise for assay development
 - Run assay against select libraries to assess performance
 - Assess full-scale screen

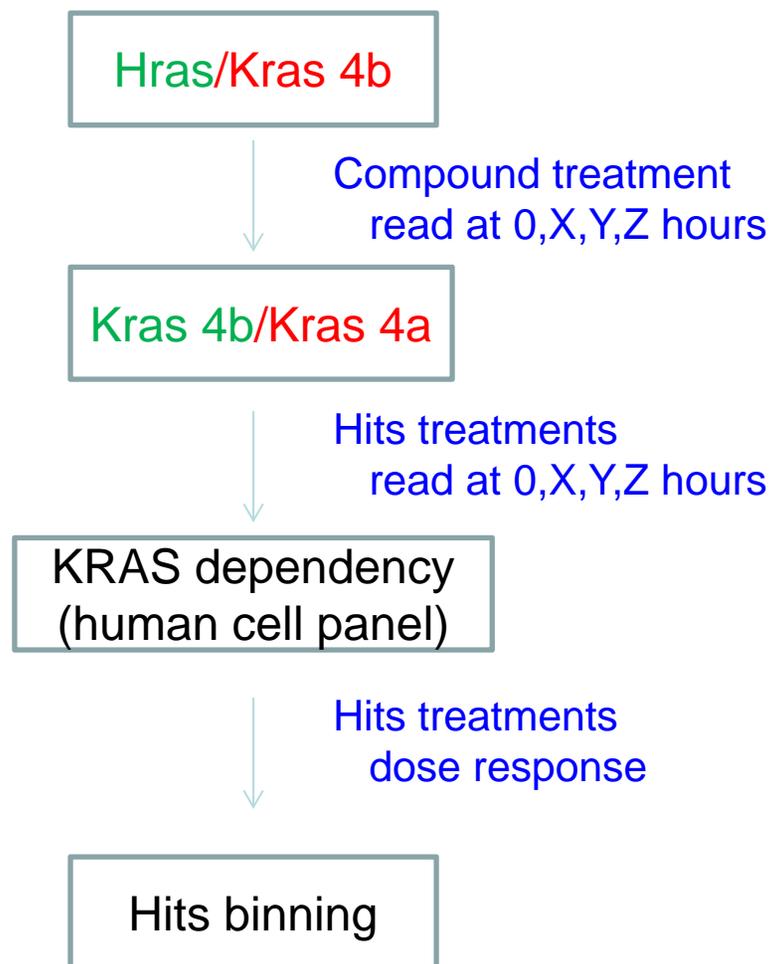
Iterative Assay Development



Several iterations of assay optimization expected before full library can be screened by NCATS.



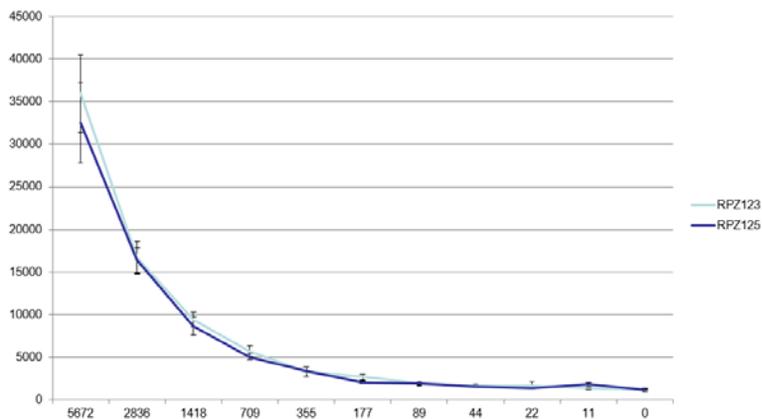
4A/4B Assay Flow Diagram



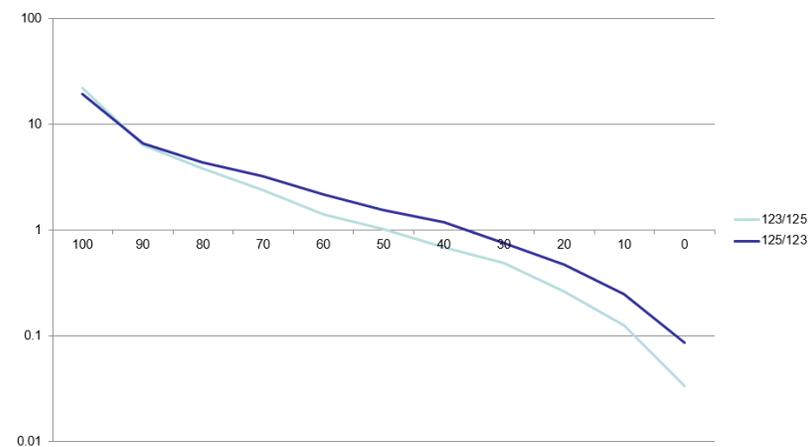
Bins

1. Hits that show differential in primary and counter screens with multiple family members and reasonable dose response
2. Hits that show differential in primary and counter screens with restricted family members and reasonable dose response
3. Hits that show differential in primary and one counter-screen

Optimizing the First Screen

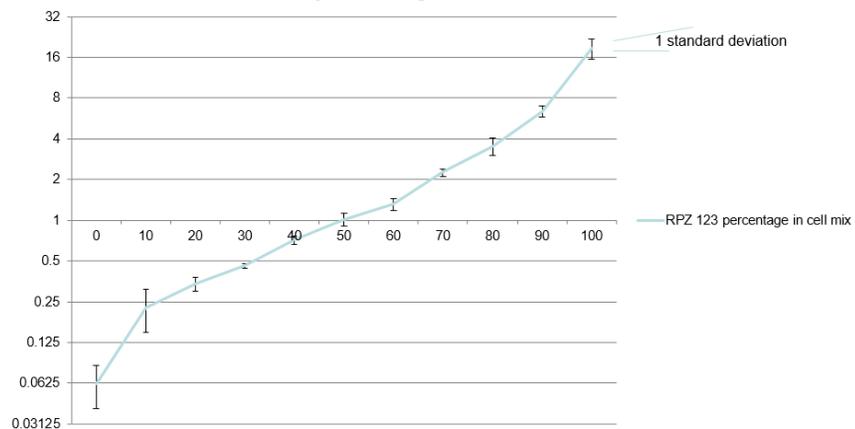


Limit of detection ~200-400 cells
Cell number determined by counting at high dilution



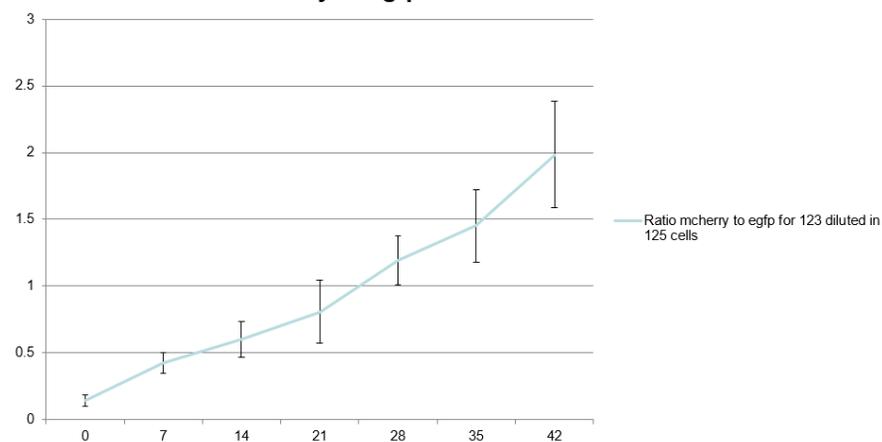
Fluor ratio is reasonably linear as a function of cell density in mixed cultures

RPZ 123 percentage in 123/125 cell mix

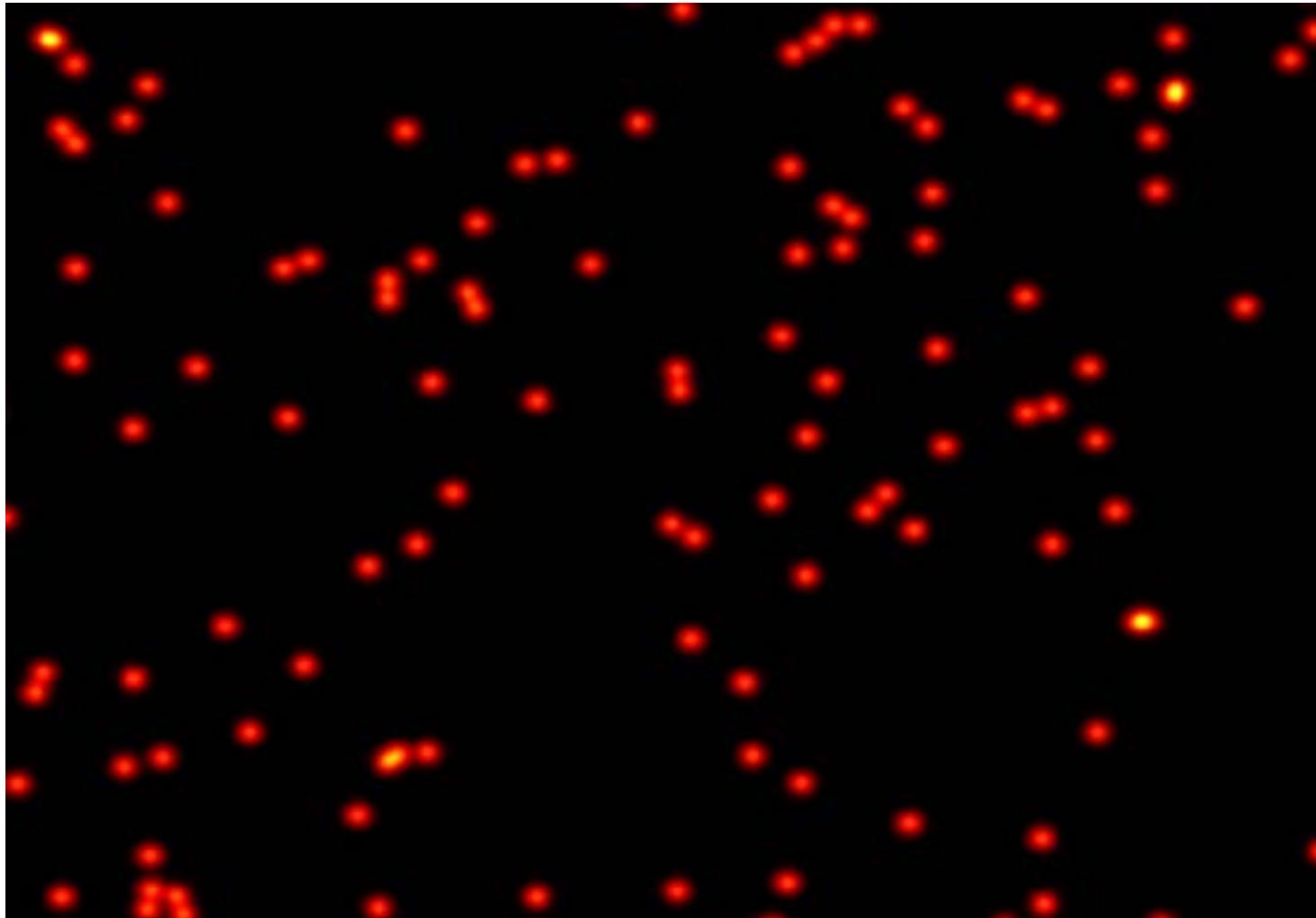


Results with manual washing/automated plate washing dislodges cells

Ratio mcherry to egfp for 123 diluted in 125 cells



Project Three: Disrupting K-Ras complexes

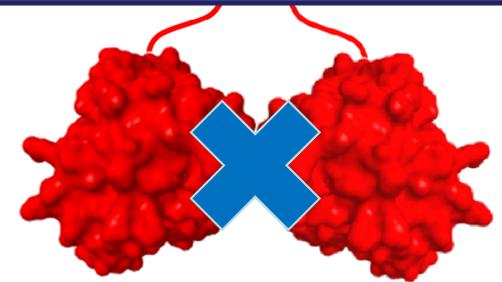


Hypothesis

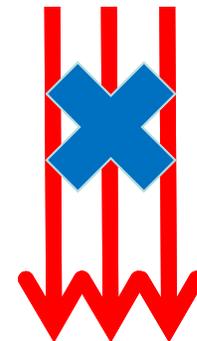


Oncogenic signaling driven by KRAS is mediated by KRAS dimers and higher order structures in the cell membrane.

Disruption of these complexes will attenuate the oncogenic signaling and therefore represents a target of drug discovery.

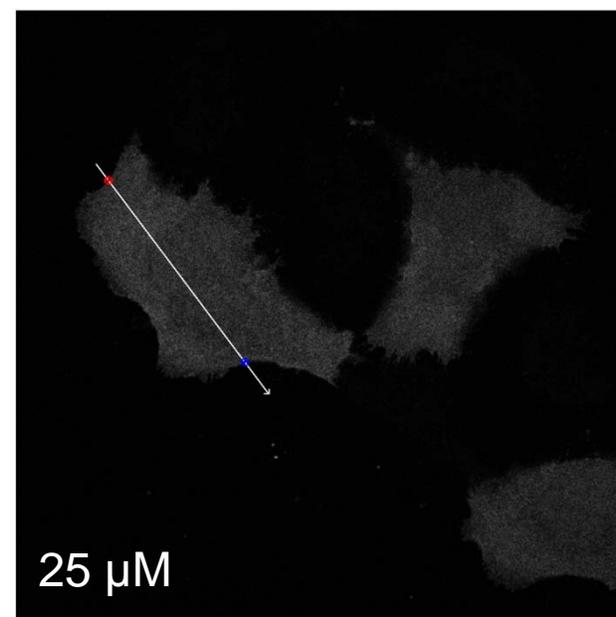
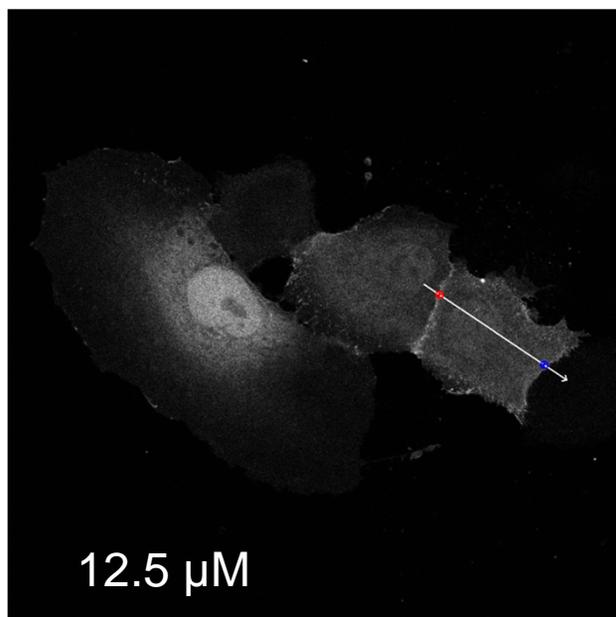
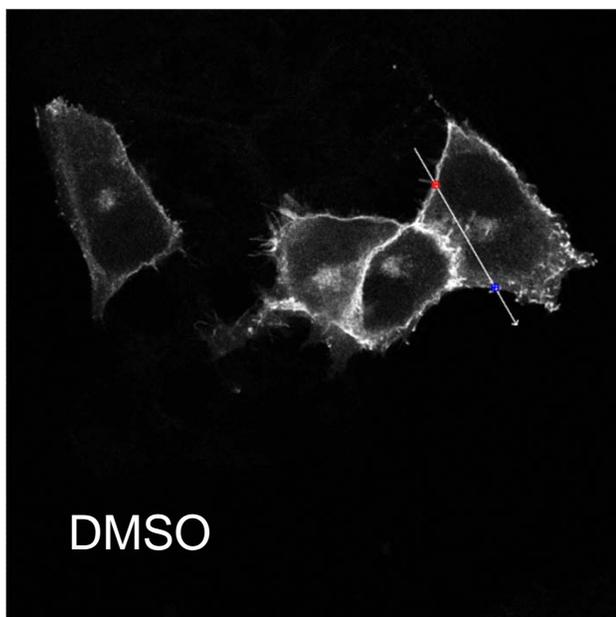


KRAS dimers and higher order complexes

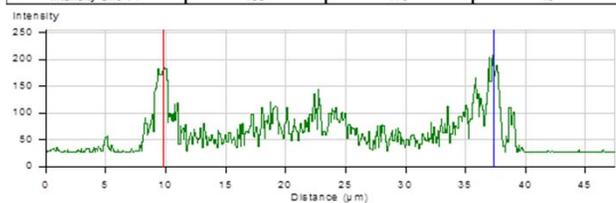


Cancer phenotype

PANC-1 cells Expressing GFP-HRas

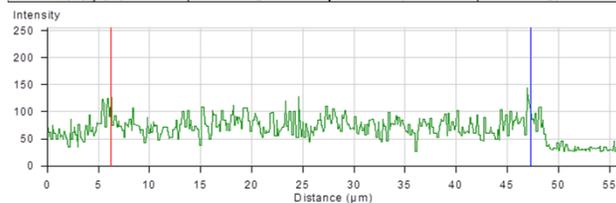


	Marker 1	Marker 2	Difference
Distance	9.817 μm	37.372 μm	27.555 μm
Intensity Ch3-T1	185	170	-15



Intensity Ch3-T1 —

	Marker 1	Marker 2	Difference
Distance	6.226 μm	47.339 μm	41.114 μm
Intensity Ch3-T1	127	107	-20



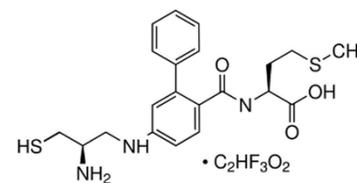
Intensity Ch3-T1 —

	Marker 1	Marker 2	Difference
Distance	4.826 μm	57.714 μm	52.888 μm
Intensity Ch3-T1	36	41	5



Intensity Ch3-T1 —

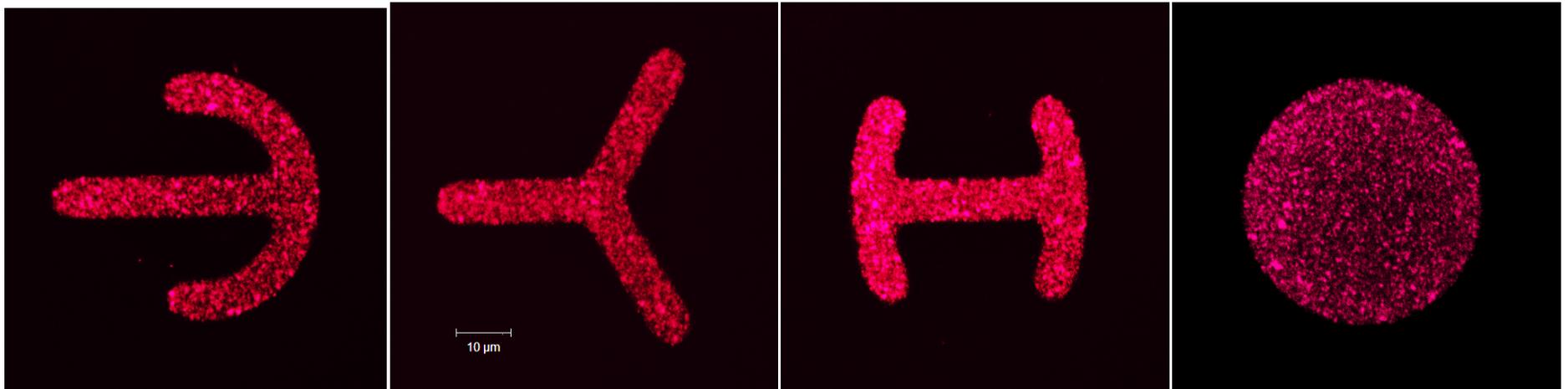
FTI-276



Micropatterns

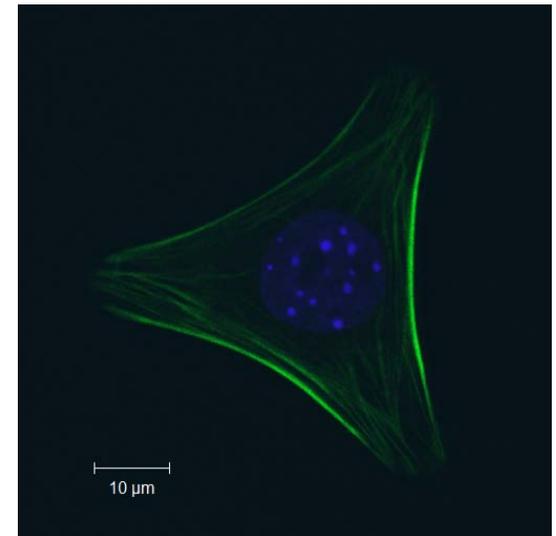
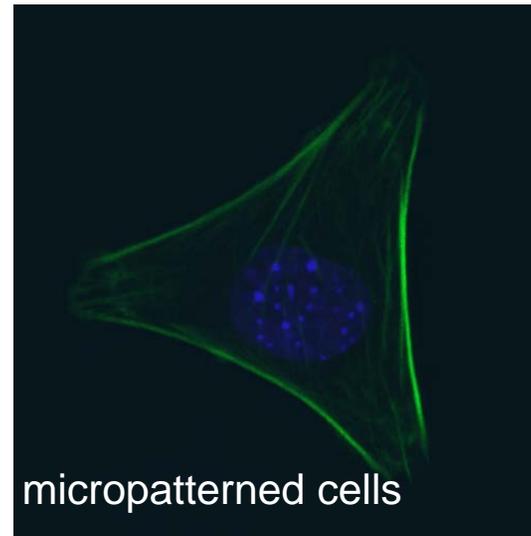
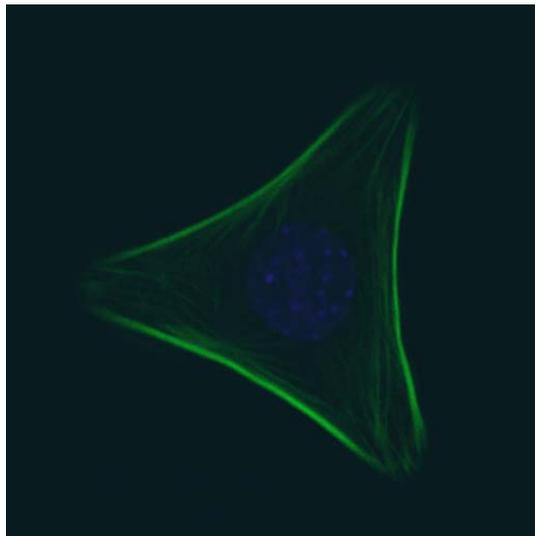
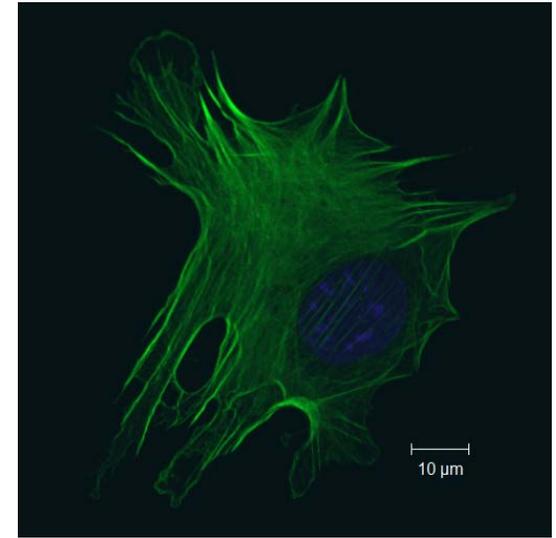
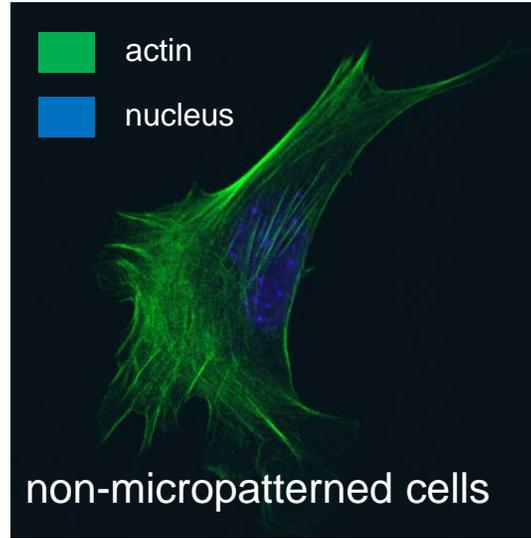
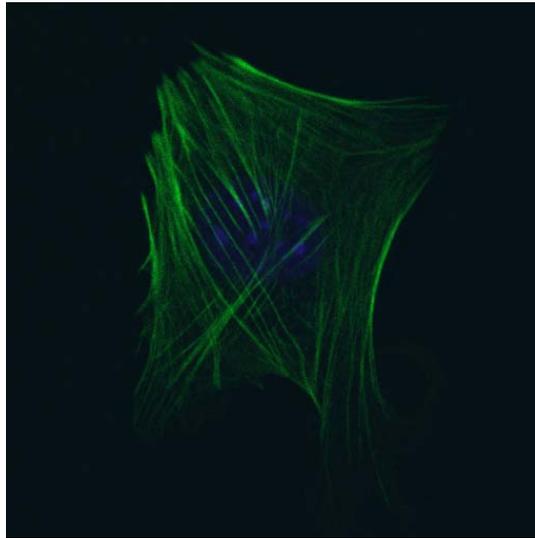


Cells on micropatterns are more homogenous in the localization of cellular structures vastly improving ability to quantify changes.



Surface of cell when spread out on micropattern @1600 μm^2

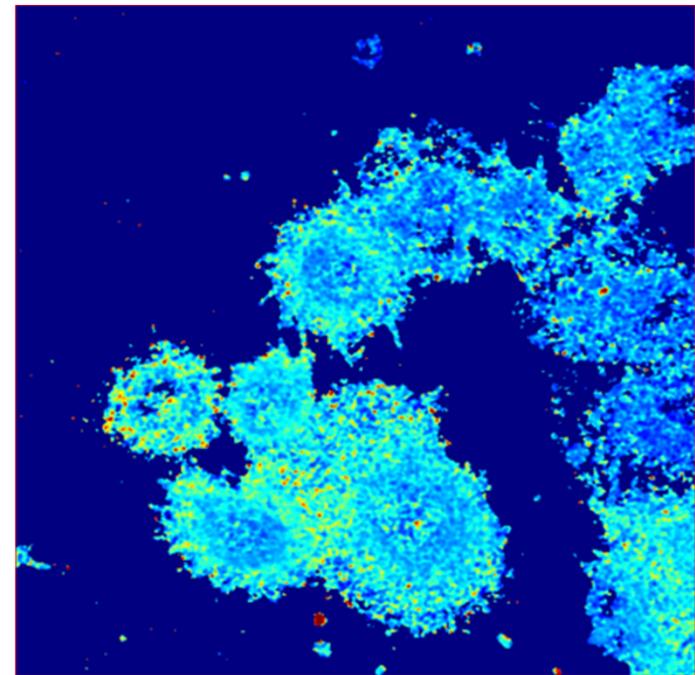
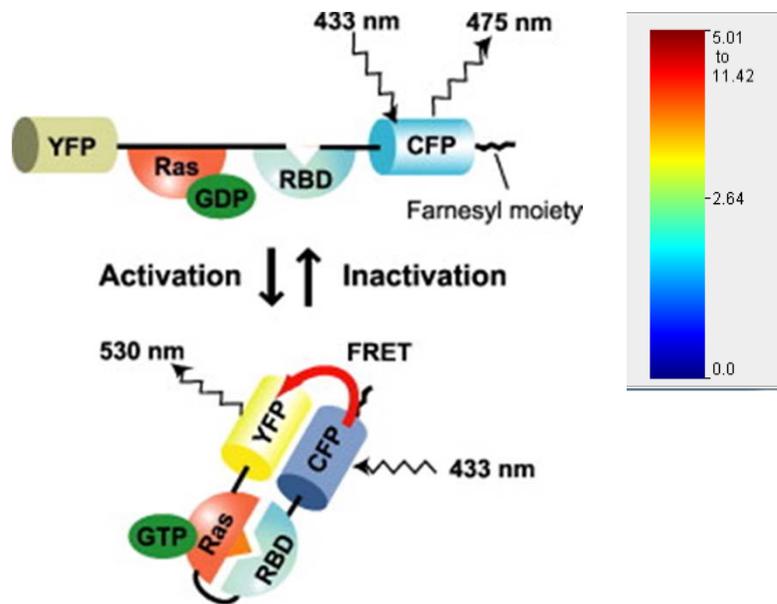
RAS-less MEFs on Micropatterns



Development of a Ras biosensor



Development of a Ras biosensor

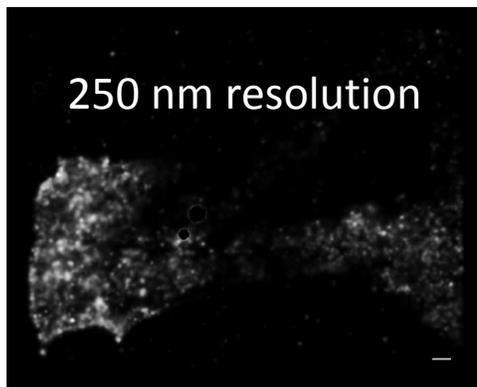
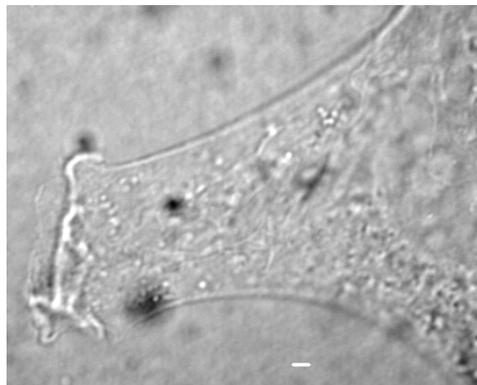


FRET signal in PANC-1 cells expressing Ras biosensor

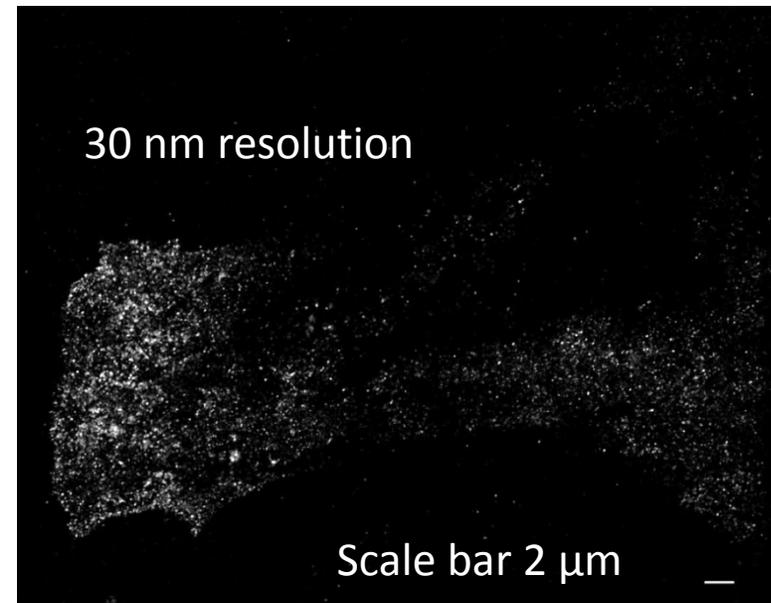
Single Molecule Imaging



Techniques like PALM and FCS provide detailed, and perhaps actionable, single molecule information about the location and persistence of KRAS complexes in cell compartments.



Bright field and TIRF image



PALM image of PA-mCherry-HRAS-G12V-MEF

Project Four: surface proteins on KRAS Cancers



- Goal: identify proteins on the surface of KRAS cancer cells for targeting nano-particles or immunotherapy, or for use as biomarkers

- Approaches:

Mass spec analysis of proteins on KRAS cancer cells

Phage display (Jim Wells et al)

Validate candidates from literature

Bioinformatics

Method 1: Cell Surface Proteome Mapping - Fractionation



1. Whole proteome MCF10A cells

Cell lysis - sonication - SCX

2. Microsomal fraction

Ultracentrifugation - SCX

3. Plasma membrane

Sucrose gradients - SCX

4. Lipid rafts

Detergent
resistant
fraction
SCX

LC-MS

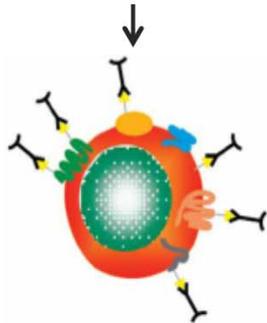
Method 2: Chemical Tagging of Cell Surface Proteins on Live Cells



sodium periodate

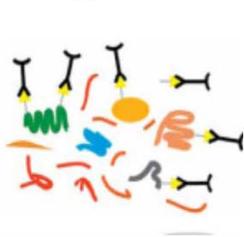


biocytin hydrazide



cell lysis

digestion



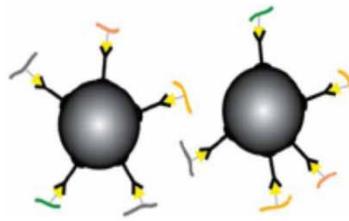
LC-MS analysis



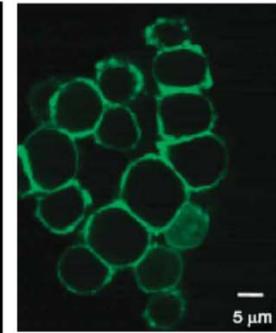
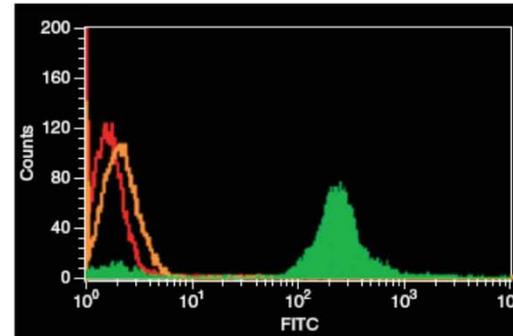
peptide release



glycosidase treatment

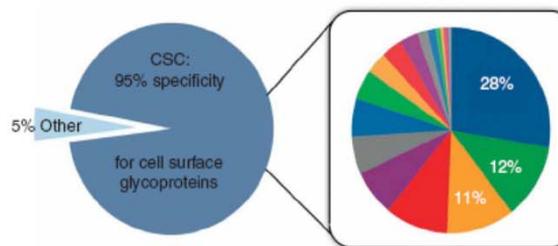


affinity enrichment



PANTHER Molecular Function:

- Receptor
- Cell adhesion molecule
- Molecular function unclassified
- Defense/immunity protein
- Transporter
- Kinase
- Signaling molecule
- Phosphatase
- Protease
- Miscellaneous function
- Hydrolase
- Transferase
- Select regulatory molecule
- Ion channel
- Cell junction protein
- Chaperone
- Lyase
- Extracellular matrix



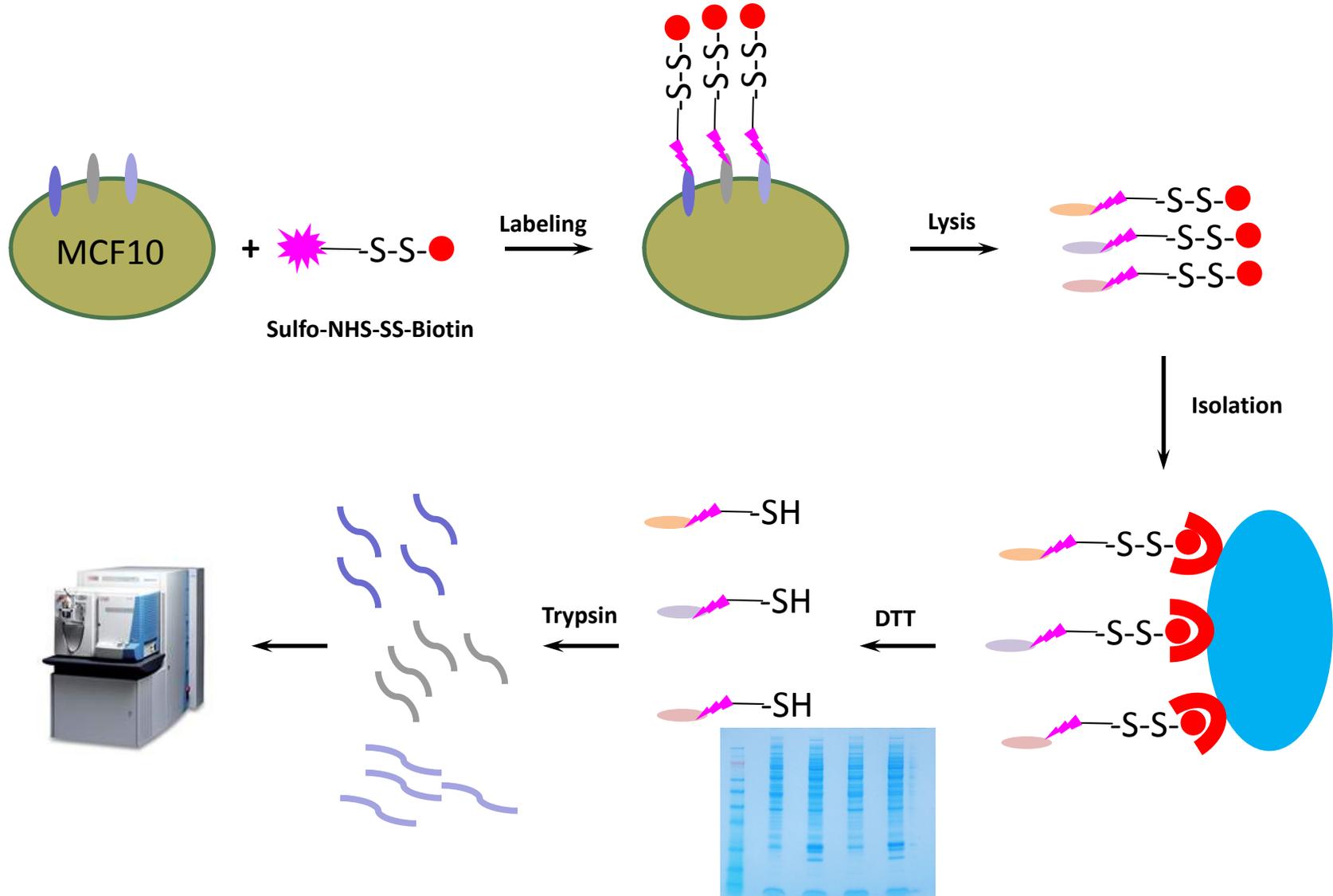
Adapted from: Wats - Nat Biotechnol. 2009; 27(4):378-86.

LC-MS analysis of MCF10A Cell Surface Proteins isolated using CSC



1	Gene	Acc No	Protein	# Peptides	# PSMs	Location
2	F5GZS6_HUMAN	(F5GZS6)	4F2 cell-surface antigen heavy chain OS=Homo sap	11	148	Plasma Membrane
3	E9PNW4_HUMAN	(E9PNW4)	CD59 glycoprotein OS=Homo sapiens GN=CD59 PE=	1	116	Plasma Membrane
4	AMPN_HUMAN	(P15144)	Aminopeptidase N OS=Homo sapiens GN=ANPEP I	14	82	Plasma Membrane
5	I3L4S8_HUMAN	(I3L4S8)	Basigin OS=Homo sapiens GN=BSG PE=4 SV=1	4	66	Plasma Membrane
6	ITB1_HUMAN	(P05556)	Integrin beta-1 OS=Homo sapiens GN=ITGB1 PE=1	9	38	Plasma Membrane
7	EMP3_HUMAN	(P54852)	Epithelial membrane protein 3 OS=Homo sapiens	1	37	Plasma Membrane
8	F5GXJ9_HUMAN	(F5GXJ9)	CD166 antigen OS=Homo sapiens GN=ALCAM PE=4	6	32	Plasma Membrane
9	CD109_HUMAN	(Q6YHK3)	CD109 antigen OS=Homo sapiens GN=CD109 PE=1	9	30	Plasma Membrane
10	B4DTU0_HUMAN	(B4DTU0)	CD166 antigen OS=Homo sapiens GN=ALCAM PE=2	6	26	Plasma Membrane
11	B7Z590_HUMAN	(B7Z590)	Cadherin-13 OS=Homo sapiens GN=CDH13 PE=2 SV	3	20	Plasma Membrane
12	S39A6_HUMAN	(Q13433)	Zinc transporter ZIP6 OS=Homo sapiens GN=SLC39	5	20	Plasma Membrane
13	E9PJC8_HUMAN	(E9PJC8)	CD151 antigen (Fragment) OS=Homo sapiens GN=C	2	18	Plasma Membrane
14	ITA3_HUMAN	(P26006)	Integrin alpha-3 OS=Homo sapiens GN=ITGA3 PE=1	7	17	Plasma Membrane
15	E9PKC6_HUMAN	(E9PKC6)	CD44 antigen OS=Homo sapiens GN=CD44 PE=4 SV	3	15	Plasma Membrane
16	CD97_HUMAN	(P48960)	CD97 antigen OS=Homo sapiens GN=CD97 PE=1 SV	4	14	Plasma Membrane
17	PTK7_HUMAN	(Q13308)	Inactive tyrosine-protein kinase 7 OS=Homo sapie	6	11	Plasma Membrane
18	FPRP_HUMAN	(Q9P2B2)	Prostaglandin F2 receptor negative regulator OS=H	5	11	Plasma Membrane
19	Q504U8_HUMAN	(Q504U8)	EGFR protein OS=Homo sapiens GN=EGFR PE=2 SV	2	10	Plasma Membrane
20	E9PB77_HUMAN	(E9PB77)	Integrin alpha-2 OS=Homo sapiens GN=ITGA2 PE=3	2	10	Plasma Membrane
21	GP126_HUMAN	(Q86SQ4)	G-protein coupled receptor 126 OS=Homo sapiens	1	9	Plasma Membrane
22	ITA5_HUMAN	(P08648)	Integrin alpha-5 OS=Homo sapiens GN=ITGA5 PE=1	5	9	Plasma Membrane
23	B7Z9S8_HUMAN	(B7Z9S8)	Sodium/potassium-transporting ATPase subunit b	2	9	Plasma Membrane
24	H0YA38_HUMAN	(H0YA38)	Sulfate transporter (Fragment) OS=Homo sapiens	2	9	Plasma Membrane

Method 3: Cell Surface Protein Labeling, Isolation and Identification



Informatics approach

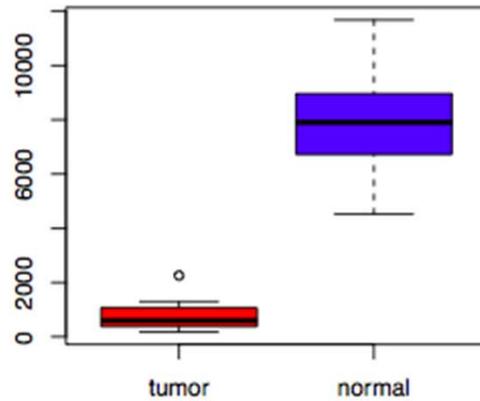


- *In-silico* identification of genes potentially differentially expressed on the surface of KRAS mutant cells relative to normal cells derived from the same tissue
- Additional potential to identify novel KRAS-associated targets and also help guide/support clinical decision making
- Approach: scan TCGA lung tumor data available for matched tumor normal pairs for differentially expressed genes

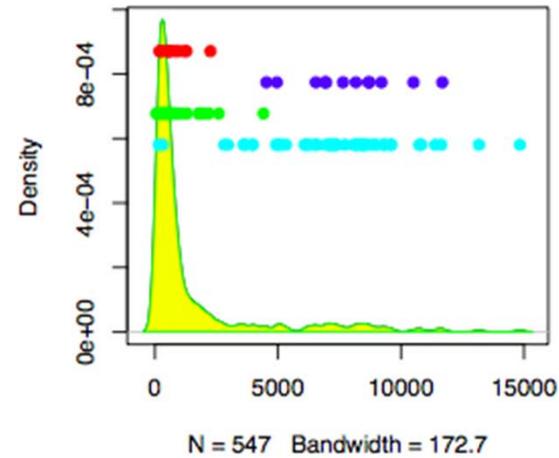
Differentially Expressed Genes from Lung Adenocarcinoma Matched Tumor Normals



Plot for FHL1 z_score=2.65

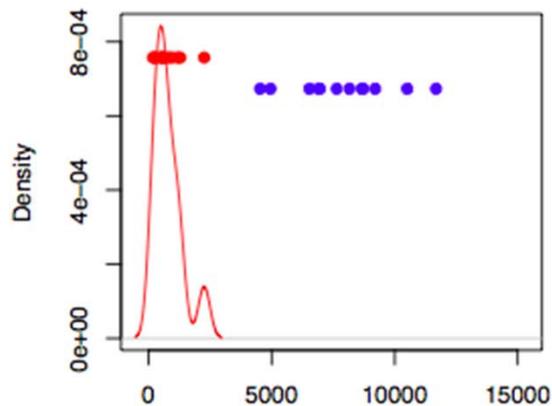


all_scores plot for FHL1

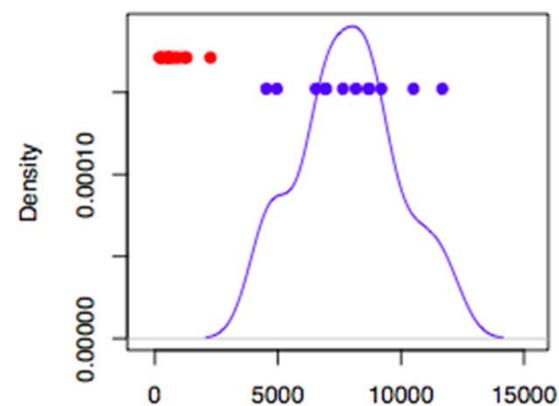


- Tumor
 - Normal
 - O.Tumor
 - O.Normal
- (n=45)

plot for tumor



plot for normal



Project Five: Synthetic lethal screens



Jan 8/9
Workshop at ATRF

Organized by Ed Harlow
Jim Hartley, Sara Hook et al

Outcome: Two potential PARS
for new synthetic lethal screens

7 pm, Session 1: Overview for the Workshop

Welcome: Dave Heimbrook

Frank McCormick, UCSF and Frederick National Laboratory

Paul Kassner, Amgen, "Clonal dominance during *in vivo* screening"

Jonathan Weissman, UCSF, "Requirements for reproducibility in global synthetic lethal screens"

Feng Zhang, Broad Institute, "Is it time to move to CRISPR?"

Thursday, January 9

Breakfast on your own, Hampton Inn

7:30 Shuttle from the Hampton Inn to the ATRF

Sessions at the ATRF

8:00 am, Session 2: "Previous synthetic lethality screens: what can we learn for future use?"

David Hancock, Cancer Research UK, London Research Institute, "Large scale RNAi based K-RAS synthetic lethal screens"

Bill Hahn, Dana Farber, "Off-target effects and saturation within large scale screens"

Anton Simeonov, Scott Martin, and Gene Buehler, National Center for Advancing Translational Sciences (NCATS), NIH, "Optimizing RNAi screening"

Michael White, UT Southwestern, "Integrating Genomic context and signaling networks into RNAi screening"

Ji Luo, NCI, "Synthetic lethal screen beyond cell viability - some preliminary parameter findings"

Group discussion

Break

Session 3, "Do 3D or *in vivo* screens get around limitations of 2D screens?"

Mike Hemann, MIT, "*In vitro* versus *in vivo* screens"

Jackie Lees, MIT, "*In vivo* screens for RAS-driven GBM"

Chris Kemp, Fred Hutchinson Cancer Research Center, "An integrated *in vivo/in vitro* approach to discover and validate druggable targets for Ras mutant cancers"

Chris Torrance, Horizon Discovery, Cambridge, UK, "3D model systems for RAS synthetic lethality screens"

Tina Yuan, UCSF, "Combinatorial gene knockdown and nanoparticle-mediated *in vivo* RNAi screening"

Garry Nolan, Stanford, Modern approaches to single cell analysis

Synthetic Lethal Screens Workshop – Conclusions – I



- Previous whole-genome RAS synthetic lethality (SL) screens were substantially underpowered
- From early results, CRISPR is probably superior to either RNAi technology (shRNA or siRNA)
- Heterogeneity matters, the more cell lines, the better
- There are indications that selections in 3D yield hits that are substantially different from 2D
- in vivo (i.e., in mice) screens require cells that form tumors very efficiently, this imposes selective pressure
- Combining knockdown or knockout of genes with inhibition of specific (druggable) pathways can reveal new susceptibilities
- Some pathway nodes are comprised of multiple redundant proteins, components of such nodes may not yield lethality or sickness that is synthetic with mutant RAS.

Synthetic Lethal Screens Workshop – Conclusions – II



- Pooled screens, in which all targeting agents are applied as a mixture to millions of cells for selection in a single container, cannot detect SL based on loss of secreted molecules.
 - This suggests that arrayed screens (target one gene at a time) using pre-validated knockdown reagents is a better approach.
- There are SL interactions that we do not understand. Cautionary to only following hits that "make sense".
- If even one KRAS mutant allele in a major human cancer could be targeted as the result of a new SL screen, thousands of lives could be saved

RAS Reference Reagents



- support internal FNL/C RTP Ras projects with qualified and standardized reagents
- generate high-quality reference reagents for the Ras extramural community (national reference reagents)

DNA clones

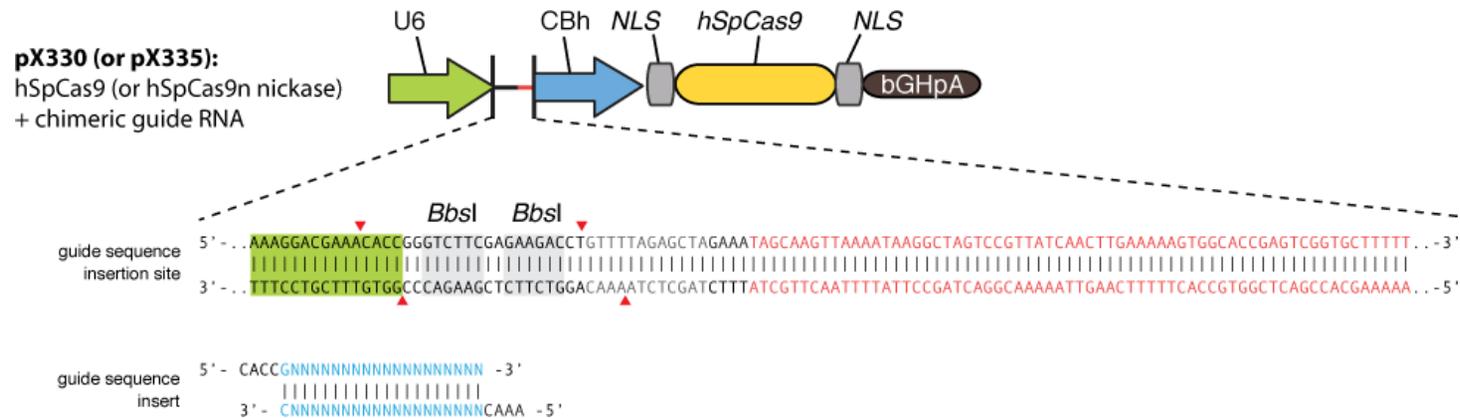
Cell lines

Viruses

Proteins

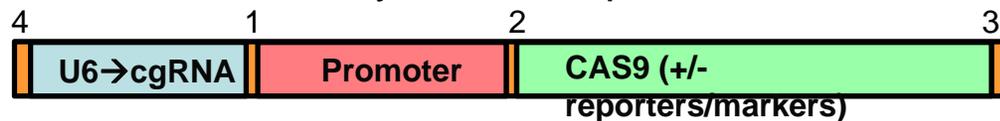
Antibodies

RAS Reference Reagents



- CRISPR detailed plans

- Initial test constructs in pX330 (GFP, NRAS, HRAS, KRAS)
- Generate Gateway-CCP compatible cassettes



- Inducible promoters, tissue-specific promoters, cell-line specific promoters
- CAS9 with T2A-GFP or T2A-Ab, or separate markers with their own promoters
- CAS9 wt and nickase versions
- FNLCR “self-inactivating” cgRNA

RAS Reference Reagents



- 109 fully sequence validated Ras Entry clones
 - Most clones are in multiple formats (ATG-closed, ATG-open, tev-closed)
 - KRAS4b (wt, G12C, G12D, G12V, G13D, 17 other mutants, 1-166)
 - KRAS4a (wt, 7 mutants)
 - HRAS (wt, 3 mutants)
 - NRAS (wt, 3 mutants)
- 36 fully sequence validated RAS pathway and RAS-related gene Entry clones
 - Open and closed full-length clones
 - for structural studies, assay development, validated clone collections

Completed	Completed	Completed	In Progress
SOS1	KNDC1	RASAL1	RGF (RalGDS)
SOS2	CALM1	RASA4	CDC37
SYNGAP1	PDE6D	RASA1 (RasGAP)	RCE1
RASGRP1	FNTA	ARAF	ICMT
RASGRP2	FNTB	BRAF	NF1 (+domains)
RASGRP3	RASGEF1a	CRAF (RAF1)	

RAS Reference Reagents



Materials generated for other RAS Projects

- Project Z
 - lentiviral constructs for wt and GFP tagged KRAS, HRAS, and NRAS
 - allele-specific shRNA constructs including inducible designs
 - materials for AAV-based gene editing
- Project 1
 - 107 protein production clones for E. coli and insect cell production
 - clones for biotin-tagged Ras for biophysical studies
- Project 3
 - mEos2/mDendra fusions of KRAS, HRAS, and NRAS for PALM
 - 3xFLAG and Halo fusions of KRAS4b and mutants for localization and P-P interaction
 - KRAS biosensors

Ras Program IFX Support



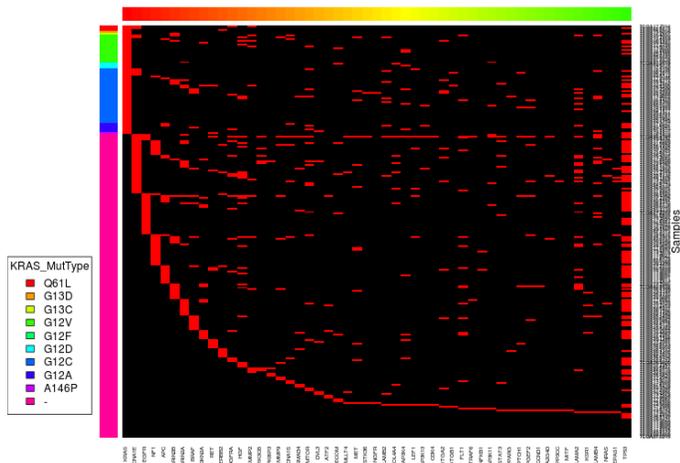
FNLCR/CRTP Ras Program Web Page Index (11/08/2013 08:05:01)

Ras Related Tools and Pages

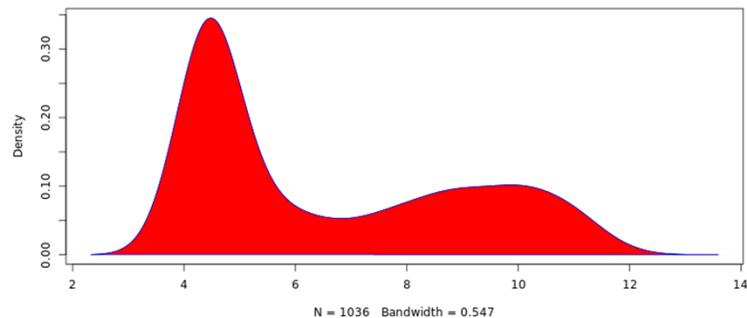
URL	Description
cell_lines.php	screen/filter through COSMIC and CCLE cell lines (file based)
cell_lines2.php	advanced screen/filter through COSMIC and CCLE cell lines (file based)
ccle_bygene.php	get expression, copy number and mutation data across cell lines (file based)
2genes.php	Select CCLE Cell Lines with Mutant Gene Pairs
copynum_bychrom.php	get CNVs by chromosome and cell line
copynum_bychrom.php	get CNVs by chromosome and cell line
copynum_bygene.php	get CNV cell lines by gene
copynum_byline.php	get CNV genes by cell_line
expr_bychrom.php	get expression by chromosome and cell line
expr_bygene.php	get expression cell lines by gene
expr_byline.php	get expression genes by cell_line
density_bygene.php	plot expression/copynum distribution
boxplot_bygene.php	plot expression/copynum by tissue
density_bygene_multi.php	plot expression/copynum dist. by tissue (all genes on same plot)



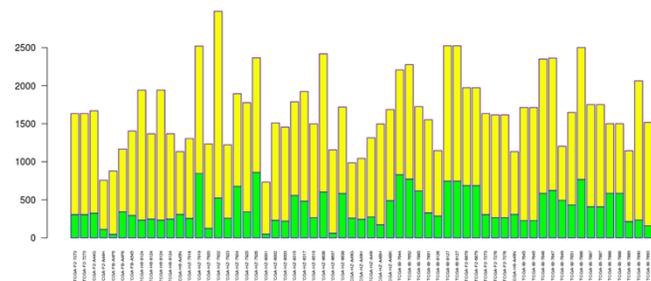
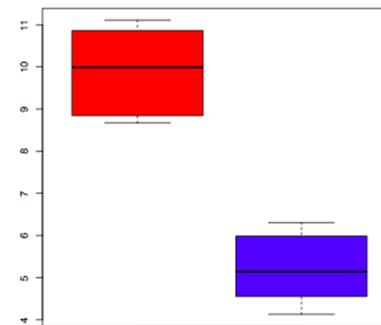
Lung_Adenocarcinoma_TCGA_KRAS_MutDataOnly_pairG



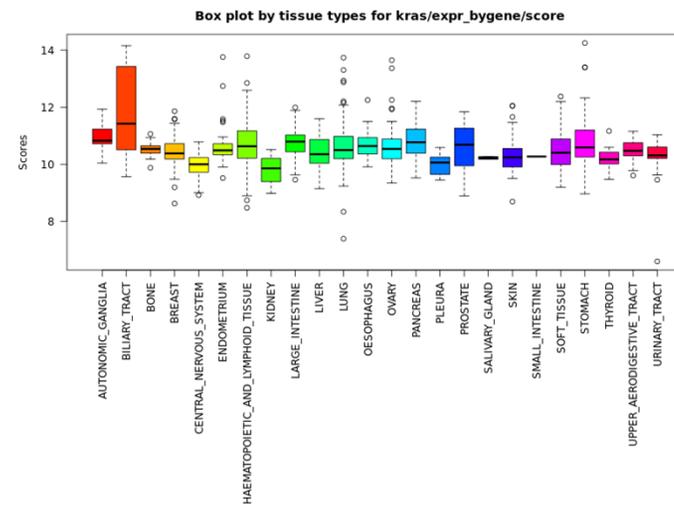
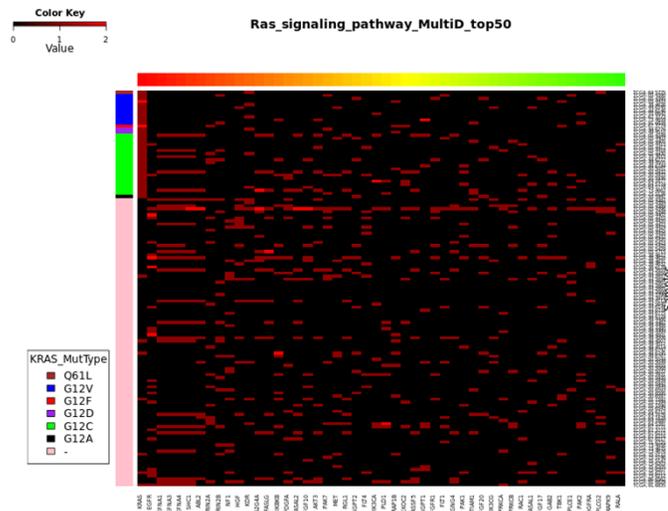
Density plot for cdh1/expr_bygene/score



Plot for CDH1 lengths=8,8



Additional IFX Applications



Genes with significant expression differences

gene	field1	sample	z_score	score
AGPS	8540_at	MCF7_BREAST	-5.51	4.11456
ZCHC11	23318_at	MCF7_BREAST	-4.1	4.27746
ACN9	57001_at	MCF7_BREAST	-4	3.23167
BTG3	10950_at	MCF7_BREAST	-3.82	5.2788
CCDC82	79780_at	MCF7_BREAST	-3.78	4.82528
LDHB	3945_at	MCF7_BREAST	-3.62	4.61737
TFCP2	7024_at	MCF7_BREAST	-3.57	4.4266
FNTA	2339_at	MCF7_BREAST	-3.52	8.51903
SIGMAR1	10280_at	MCF7_BREAST	-3.39	6.66514
TMEM123	114908_at	MCF7_BREAST	-3.29	9.40265
HOOK3	84376_at	MCF7_BREAST	-3.22	4.49424
OSBPL3	26031_at	MCF7_BREAST	-3.16	4.26013
NUPL2	11097_at	MCF7_BREAST	-3.15	6.12238
HSD17B11	51170_at	MCF7_BREAST	-3.1	4.60565
SNUPN	10073_at	MCF7_BREAST	-2.98	7.72223
AKR1B1	231_at	MCF7_BREAST	-2.95	4.27746
FAM135A	57579_at	MCF7_BREAST	-2.91	4.7803
IGF2BP3	10643_at	MCF7_BREAST	-2.9	4.13068
ZNF655	79027_at	MCF7_BREAST	-2.89	4.12727

```
./cell_lines2.pl genes=KRAS:G12D:homo tissues= cell_lines= sublists= printflag=terse
```

Cell Lines Matching Criteria:

```
cell_lines=
genes=KRAS:G12D:homo
tissues=
histo=
sublists=
```

Match for ASPC-1:

```
COSMIC: No Mutant Information Available
CCL: pancreas carcinoma ductal_carcinoma [was AsPC-1]
```

mutations:

```
KRAS:G12D:homo(74/0):Misense_Mutation:SNP
```

Match for AU-565:

```
COSMIC: large_intestine carcinoma adenocarcinoma [was C2BBe1]
```

mutations:

```
APC G1367* homo
BRAP V600E homo
CDH1 ? homo
CDKN2A 0? homo
CDKN2A 0? homo
CDKN2A 0? homo
CDKN2A ? homo
CDKN2A ? homo
CDKN2A L78fs*41 homo
CDKN2C 0? homo
CDKN2C 0? homo
CDKN2a(p14) 0? homo
CDKN2a(p14) 0? homo
CDKN2a(p14) ? homo
CDKN2a(p14) ? homo
CDKN2a(p14) ? homo
CDKN2a(p14) ? homo
CDKN2a(p14) B134fs*41 homo
FBXW7 ? homo
FBXW7 R465C het
FBXW7 R465H het
HRAS Q61L homo
```