NATIONAL HUMAN GENOME RESEARCH INSTITUTE Division of Intramural Research

Towards Deciphering The Genetics Landscape in Melanoma

Yardena Samuels, Ph.D.

NHGRI/NIH

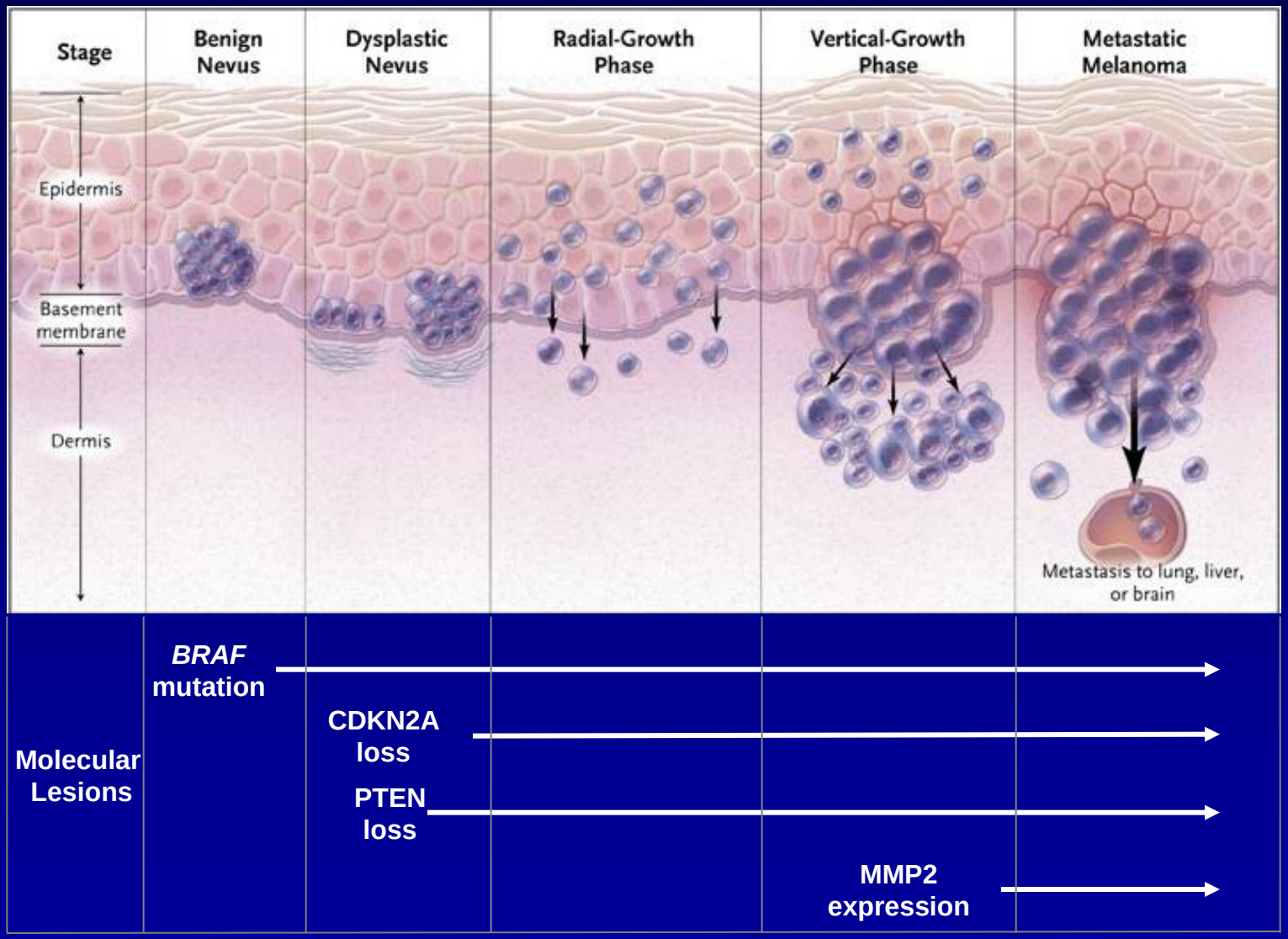
ESMO Congress, Vienna

September 30th 2012

Disclosure slide

- I have no Conflicts of Interest to declare

Histologic Progression of Melanocyte Transformation



Adapted from Miller AJ and Mihm MC, NEJM, 355:51-65, (2006)

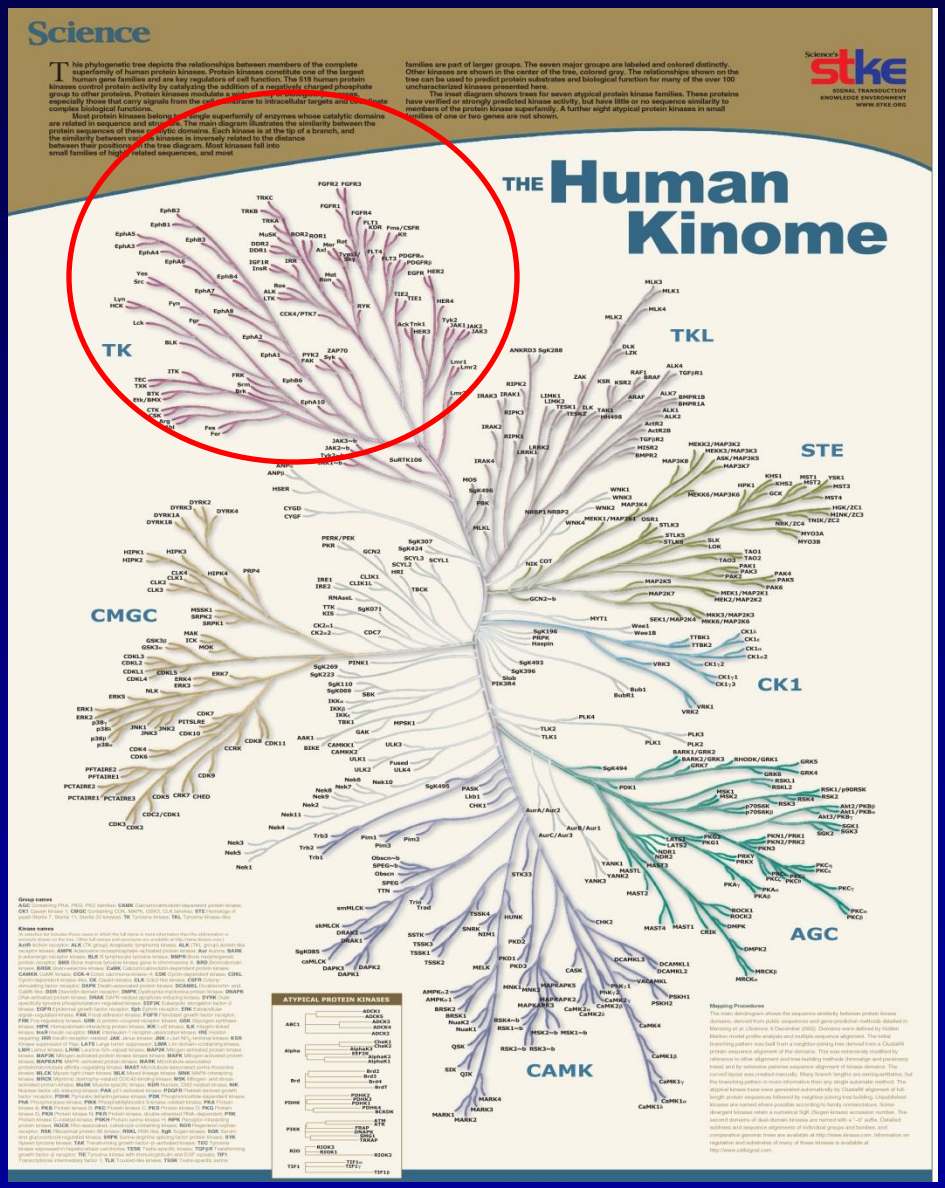
Our Goals

- Mutational analysis of the melanoma genome
- Functional analysis of the most highly mutated genes
- Translation of the genetic and functional data into the clinic

Tumor Bank Establishment

	Dr. Rosenberg National Cancer Institute	Dr. Gershenwald MD Anderson	Dr. Robinson Colorado Cancer Center
Metastatic tumor DNA	120	32	40
Matched normal DNA	Yes	Yes	Yes
OCT blocks	Yes	Yes	Yes
Matched cell line	Yes	No	No
Matched RNA	Yes	No	No
Matched protein lysate	Yes	No	No
Clinical Information	Yes	Yes	Yes

Analysis of the Tyrosine Kinome



Manning et al., *Science* 298: 1912-1934 (2002)

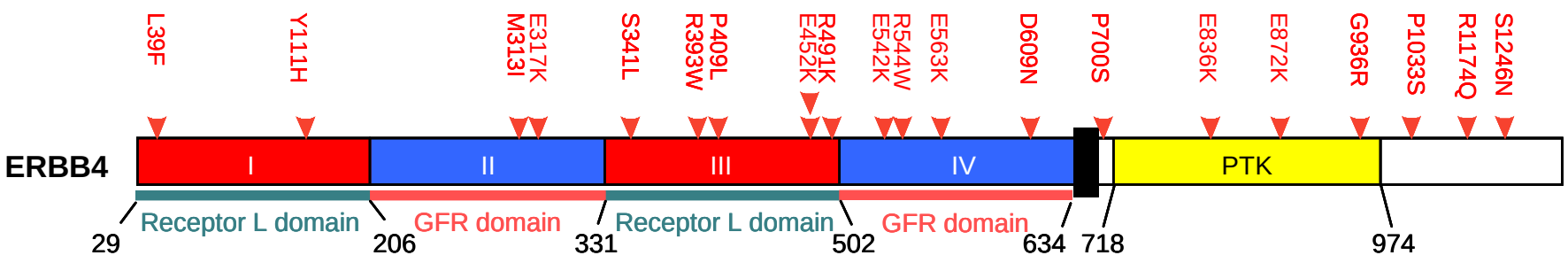
Somatic Mutations Found in ERBB4 (19% in total)

LETTERS

nature genetics

Analysis of the tyrosine kinome in melanoma reveals recurrent mutations in *ERBB4*

Todd D Prickett[1], Neena S Agrawal[1], Xiaomu Wei[1], Kristin E Yates[1], Jimmy C Lin[2], John R Wunderlich[3], Julia C Cronin[1], Pedro Cruz[4], NISC Comparative Sequencing Program[5], Steven A Rosenberg[3] & Yardena Samuels[1]

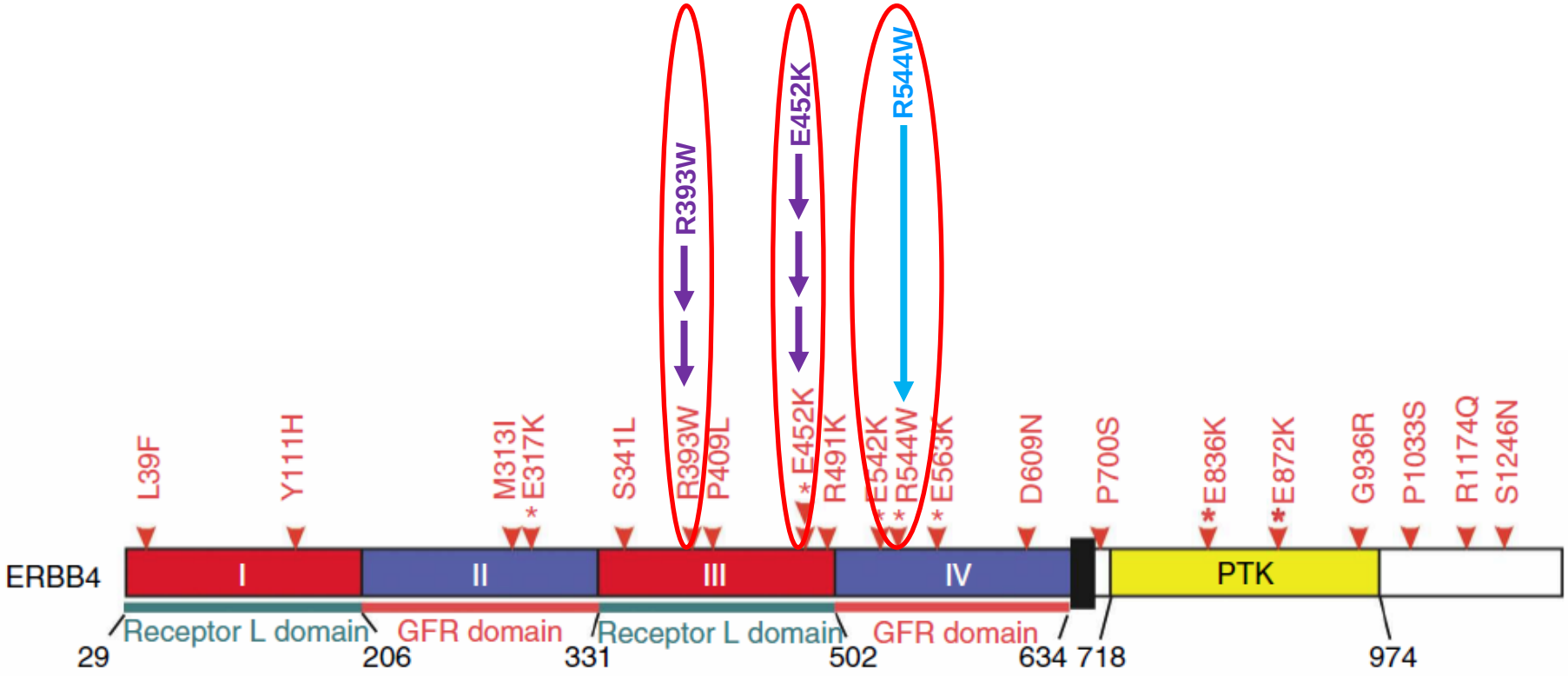


Somatic Mutations Found in ERBB4

Samuels *Nature Genetics* (15/79; 19%)

Chin *Cell* 2012 (18/121; 15%)

Hayward *Nature Genetics* (2/8; 25%)



ERBB4 ‘Driver’ Statistics

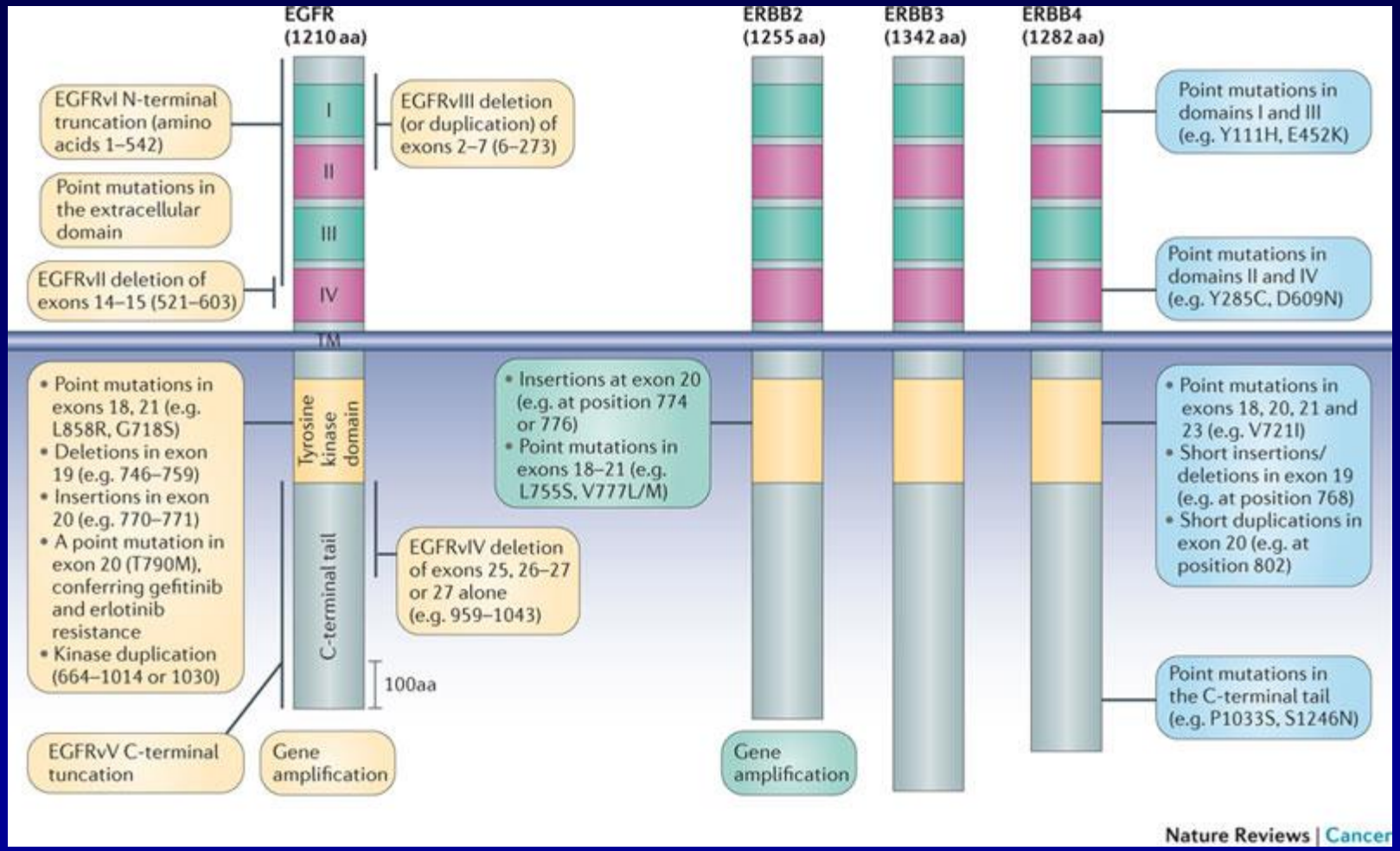
Non-synonymous: Synonymous ratio
Tyrosine Kinome paper: *Nature Genetics*, 41: 1127-1132 (2009)

24:3 (p<0.01)

Non-synonymous: Synonymous ratio
Exome paper: *Nature Genetics*, 43:442-446 (2011)
12:1 (p<0.03)

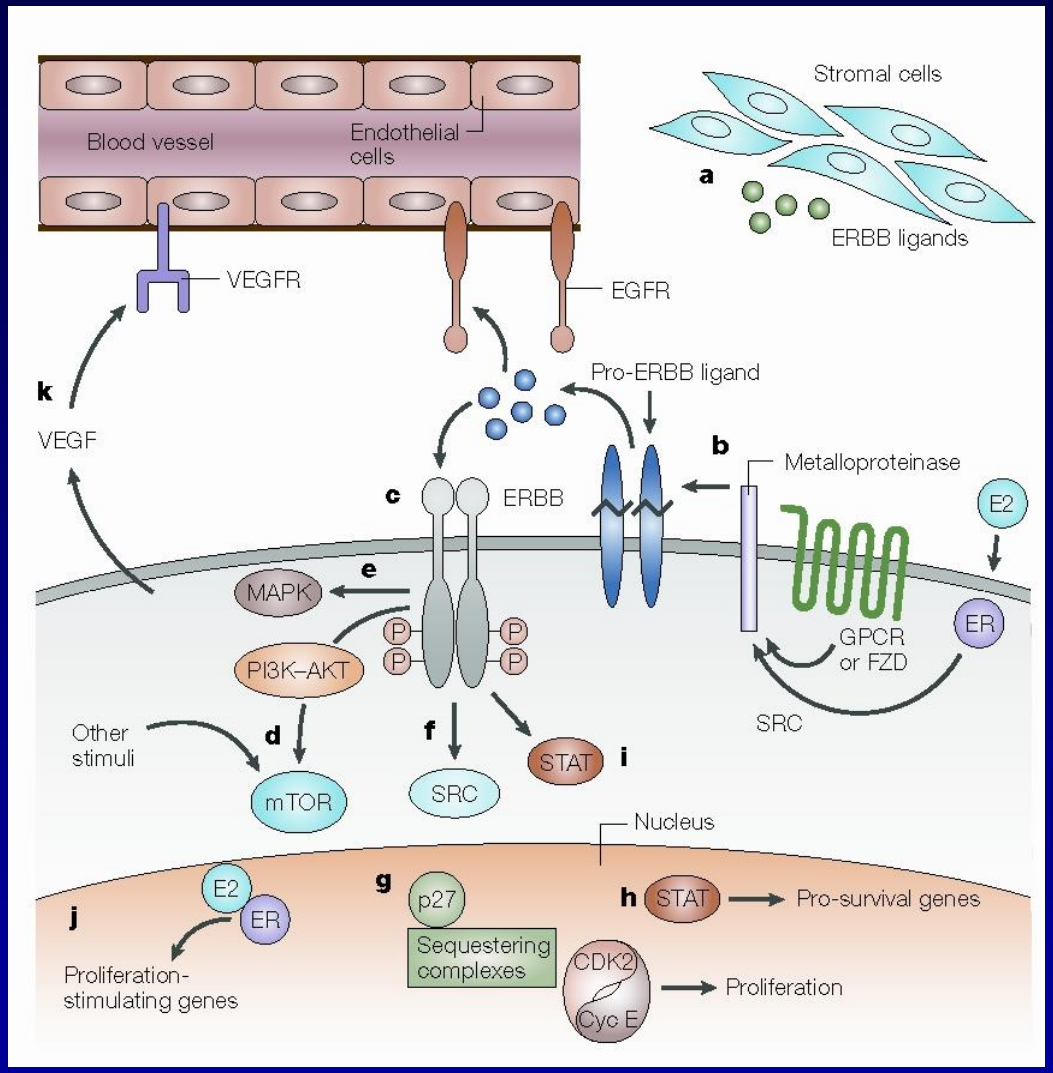
Mutations above background mutation rate
Exome paper: *Nature Genetics*, 43:442-446 (2011)
4.99E-13

ERBB Mutations in Cancer



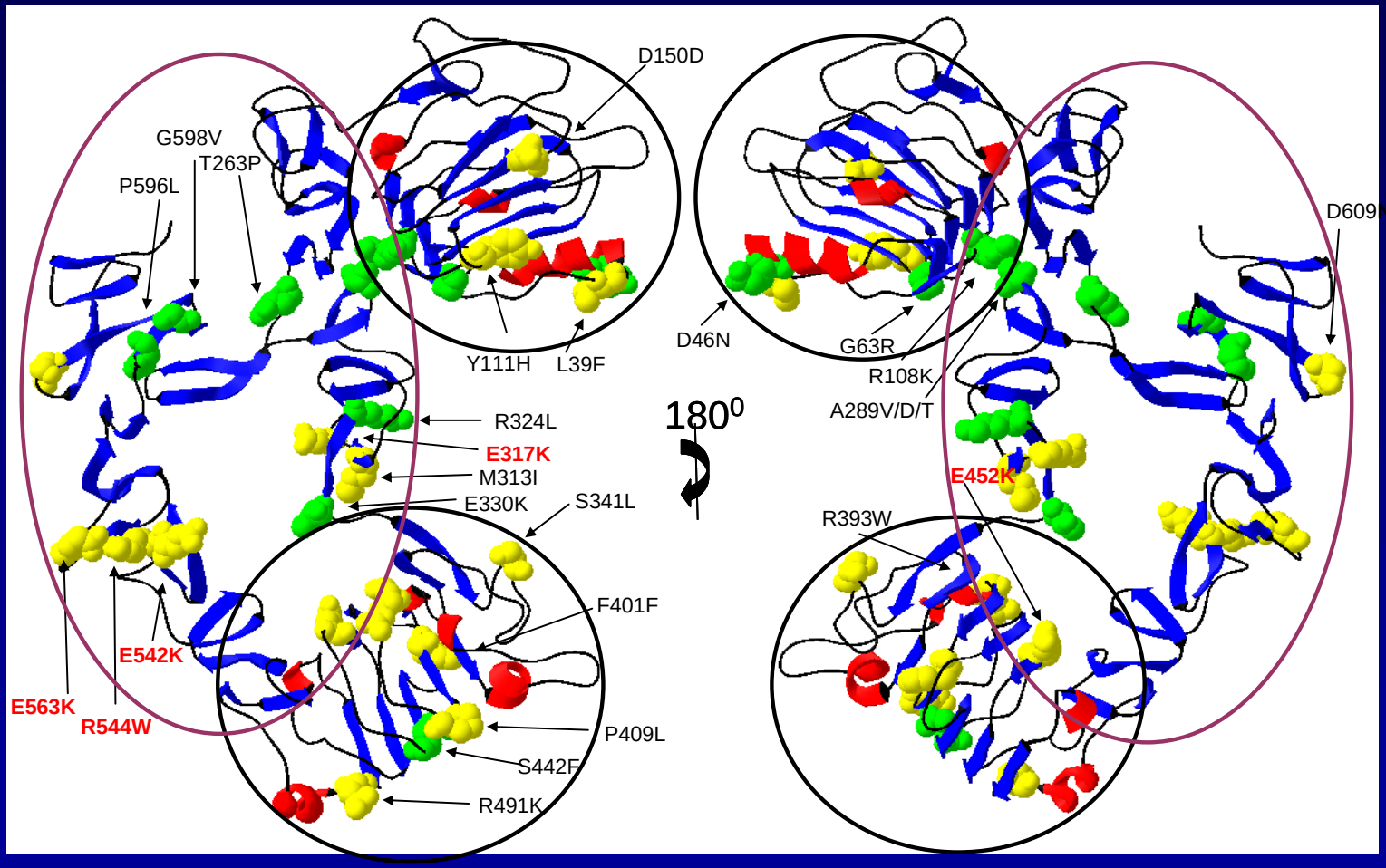
Yosef Yarden & Gur Pines Nat Rev Can 12, 553-563 (2012)

ERBB Signaling Pathways



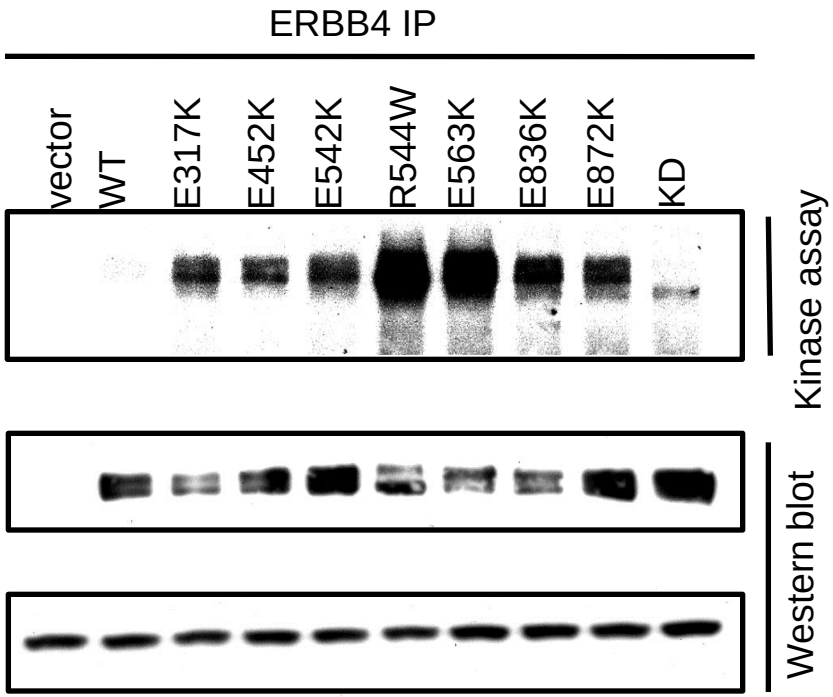
Adapted from Hynes NE and Lane HA, *Nature Reviews-Cancer*, 5:341-354 (2005)

3D-Structure of Extracellular Region of ERBB4



Prickett *et al.*, *Nature Genetics*, 41: 1127-1132 (2009)

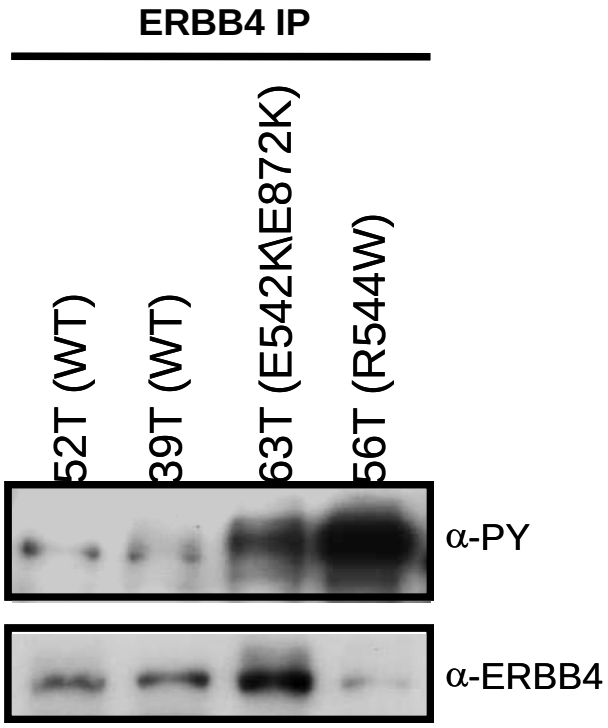
ERBB4 Mutants Have Increased Basal Activation



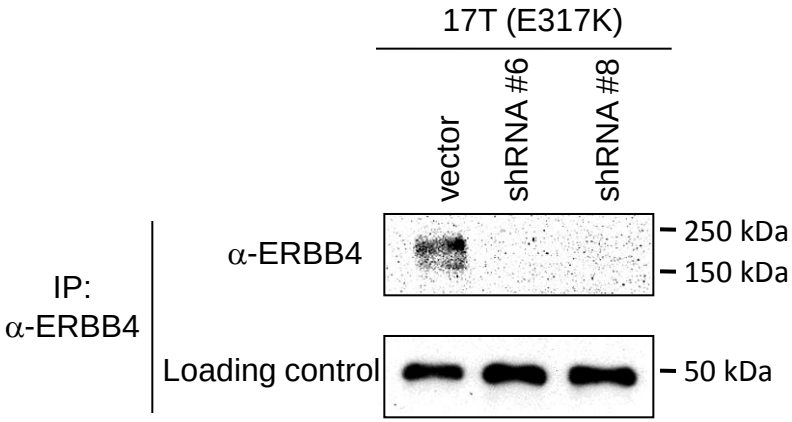
HEK 293T cells
KD-Kinase dead (K751M)

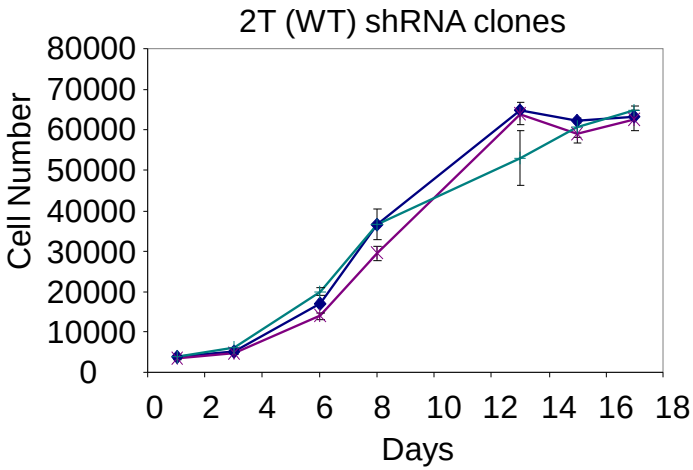
Prickett *et al*., *Nature Genetics*, 41: 1127-1132 (2009)

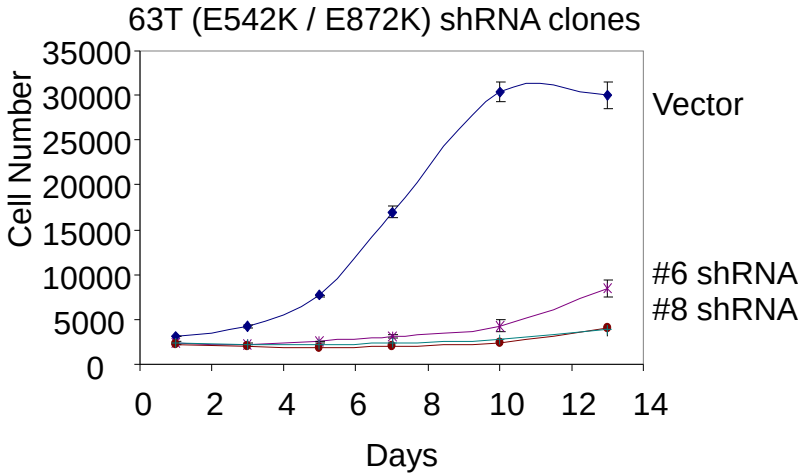
Mutant ERBB4 Displays Increased Basal Activity in Melanoma Cells



Expression of Mutant ERBB4 Provides an Essential Cell Survival Signal in Melanoma

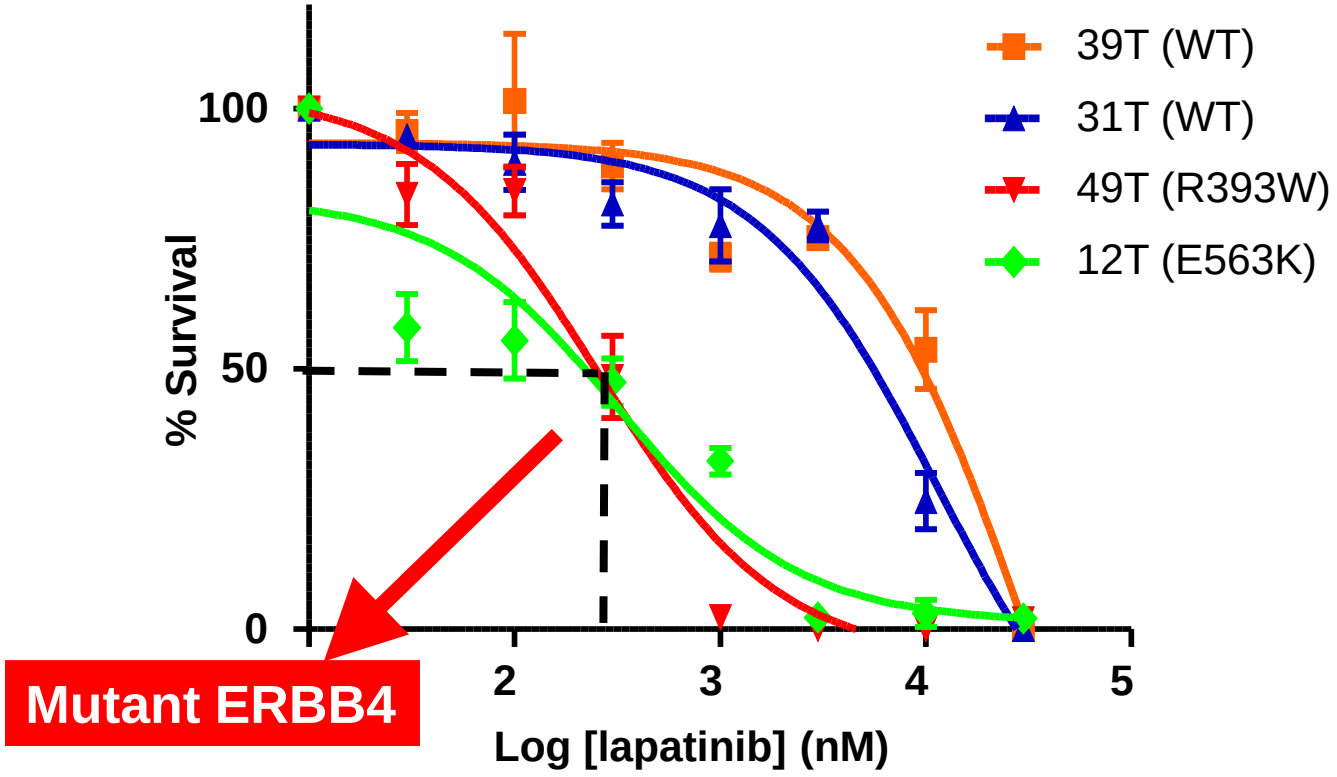






Prickett T, *Nature Genetics*, 41: 1127-1132 (2009)

Mutant ERBB4 Sensitizes Melanoma Cells to Lapatinib



1 is 10 nM lapatinib

Prickett T, *Nature Genetics*, 41: 1127-1132 (2009)

Clinical Application of the ERBB4 Study

Hypothesis: ~ 20% of current melanoma patients harbor *ERBB4* mutations. Exposure of melanoma cells harboring these mutations to lapatinib will result in cell growth inhibition.

CLIA certified ERBB4 sequencing: Paul Meltzer (NCI)

Dose: 500 mg orally twice daily # of patients: 25

Lapatinib provided by Cancer Therapy Evaluation Program (CTEP)

Multi-site clinical trial involving:

National Cancer Institute

Memorial Sloan Kettering

Clinical trial samples (NCI; MSKCC) (9/35; 25%)

ERBB4 Detection in Patient Sera

All mutant samples had detectable levels of ErbB4 ectodomain in the serum. Median of 21.2. ng/ml.

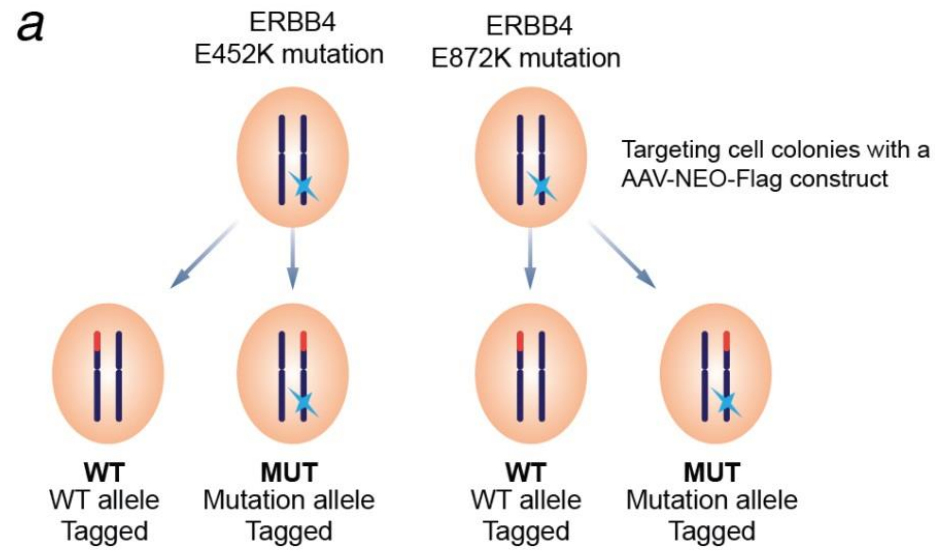
In comparison, in 30 healthy individuals the median is 7.8 ng/ml.

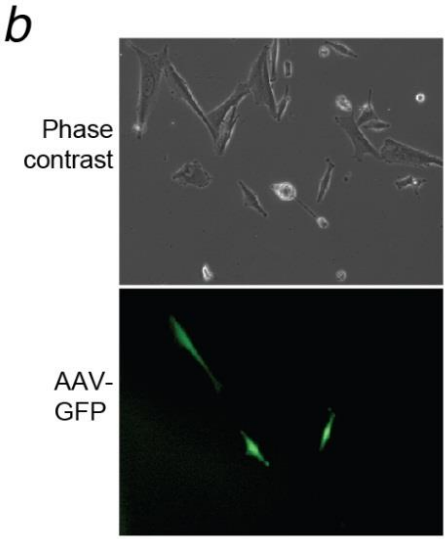
T-test-highly significant difference for the two groups ($p<4.14E-05$)

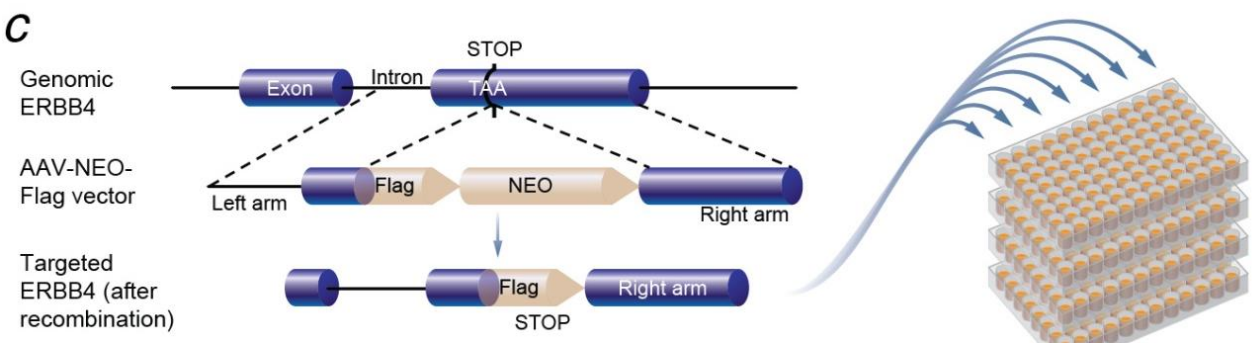
Klaus Elenius

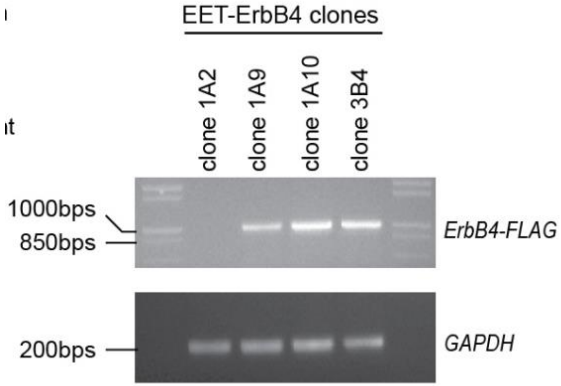
ERBB4 Interacting Proteins Strategy

The Endogenous Epitope Tagging (EET) Approach



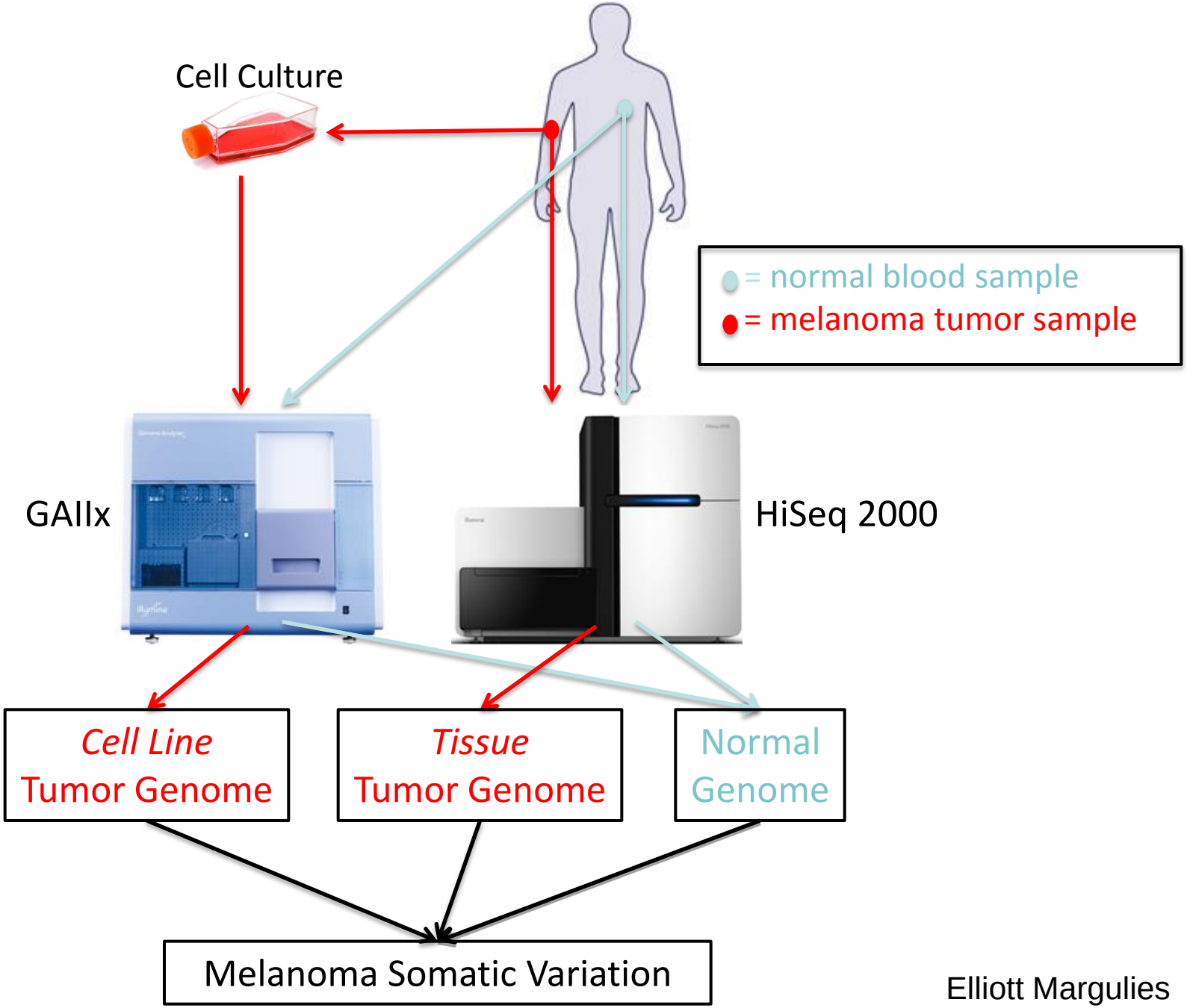






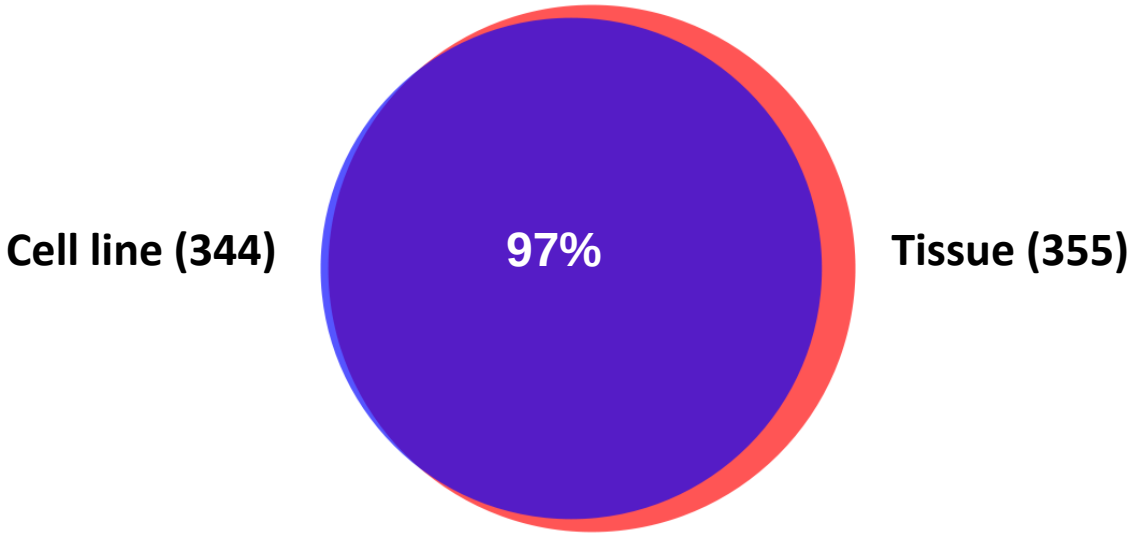
Melanoma Whole Genome Sequencing

Elliott Margulies
Steven Parker



Elliott Margulies

Intersection of Fresh Tumor and Matched Cell Line

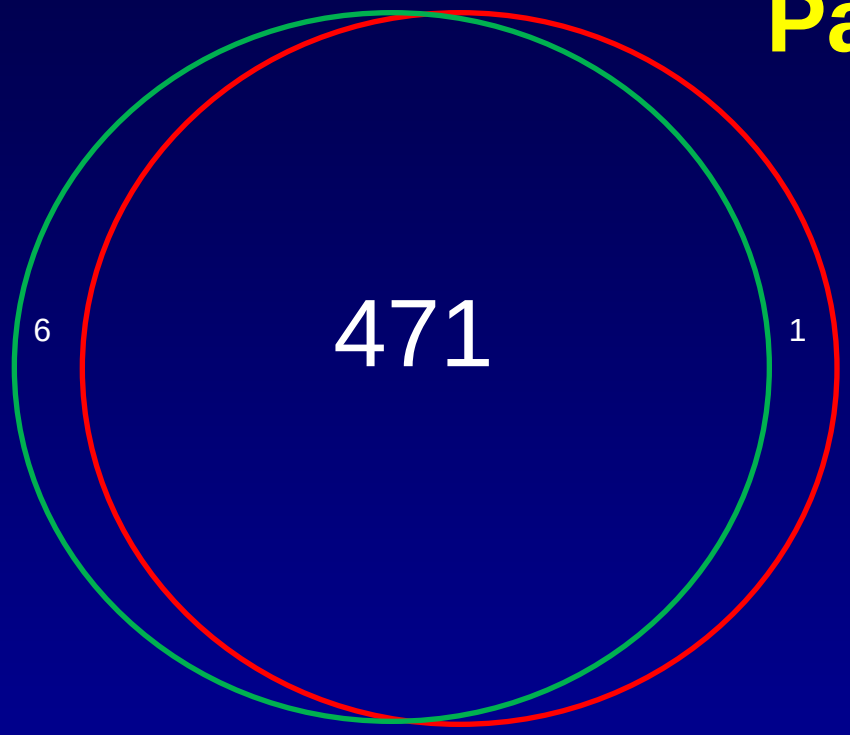


Intersection of non-synonymous and nonsense somatic variants in CDS

However, copy number variations were less concordant:
78.9% of tissue CNVs overlap with cell culture CNVs

Parker et al, *PLoS Genetics 2012*

Comparative Exome Sequencing of Melanoma Lesions Derived from the Same Patient



98% of variants seen in both Metastases

96% of variants seen in both Metastases

However, copy number variations were less concordant: 89% and 65% similarities between the two metastases

Gartner et al, *BMC Genomics*, Accepted (2012)

Implications

Though just a small pilot of 2 sets these findings indicate the potential to sequence a single metastasis from an individual to identify drivers and determine treatment strategy.

Whole Exome Discovery Screen

Exome capture sequencing of **14** untreated melanoma samples and their matched normal
180x coverage, 90% bases with high quality genotype calls

↓

Number of potential somatic mutations: **5161**

↓

Assemble sequence data (genome build hg18) and filter putative somatic mutations

↓

Number of mutations with a MPG/Coverage ratio>=0.5: **4226**

↓

Missense/ nonsense/ splice site mutations: **2813**
Insertions/Deletions: **27**
Synonymous mutations: **1386**

Nonsynonymous:synonymous ratio 2:1

The Challenge in Cancer Genomics
‘Passengers’ versus ‘Drivers’

- **Statistics**

  - Recurrantly mutated genes: “Hotspots”
  - Highly mutated genes
  - Nonsynonymous: synonymous ratio
  - Mutations above background mutation rate

- **Bioinformatics**

- **Functional studies**

Validation Screen

Search for
recurrent “Hotspot”
mutations

↓

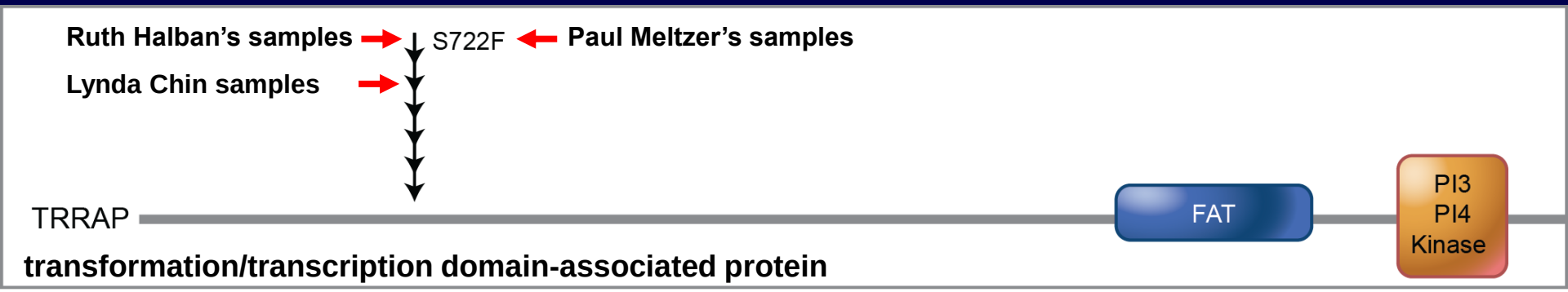
9 novel genes with
recurring mutations

↘

Validate mutated genes in
Additional tumors

Discovery (n=14)
Prevalence (n=70)
Validation set 1-Colorado Cancer Center (n=39)
Validation set 2 –MD Anderson (n=32)
Commercial cell lines (n=12)

Distribution of novel nonsynonymous recurrent mutations



The likelihood for the occurrence of 6 identical mutations is approximately $5x10^{-20}$

- A 433 kDa nuclear protein with homology to the ATM/PI-3 kinase family
- Functions as part of a histone acetyltranferase complex
- Disruption of TRRAP causes defects in cell cycle progression

Wei et al, *Nature Genetics*, 43:442-446 (2011)

Validation Screen

Search for
recurrent “hotspot”
mutations

Search for
highly mutated
genes

9 novel genes with
recurring mutations

16 highly mutated genes
(binomial p value < 0.05;
mutated in > than 2 discovery samples

Validate mutated genes in
Additional tumors

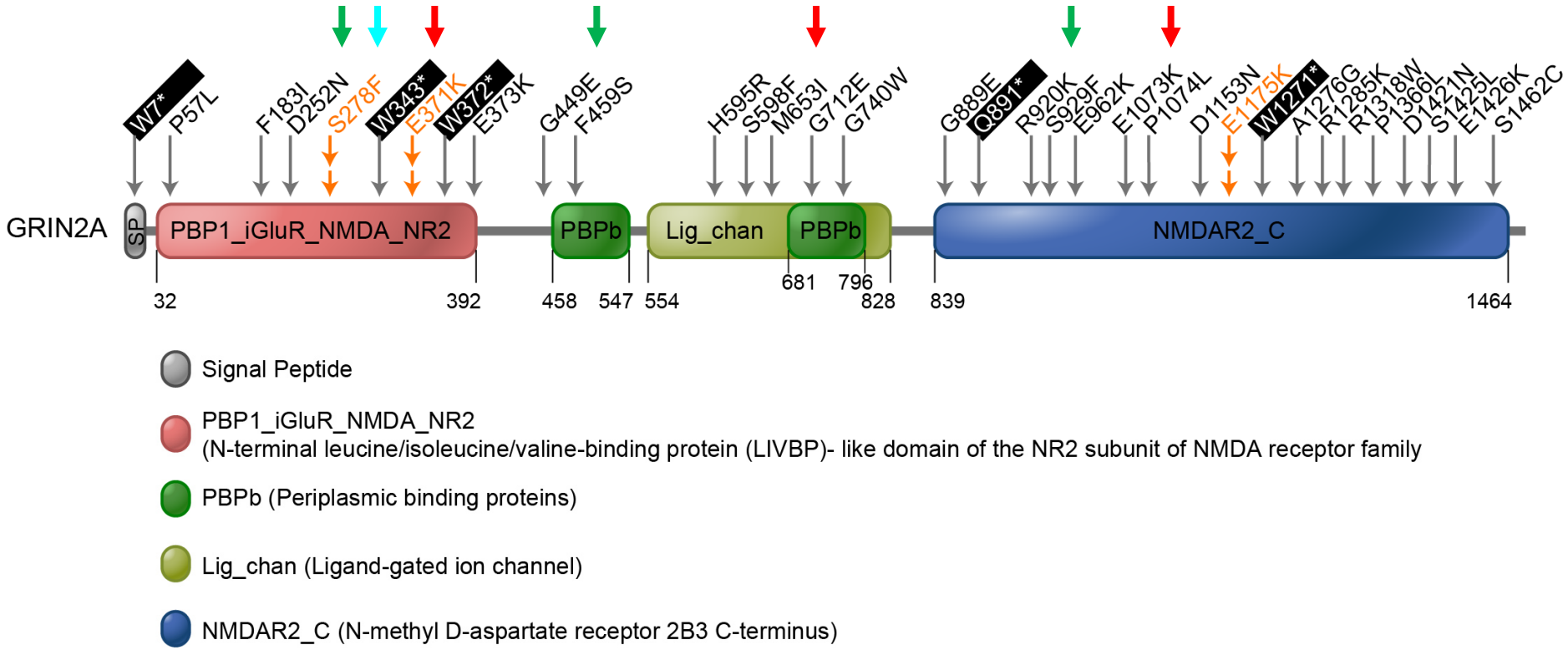
Discovery (n=14)
Prevalence (n=38)

Whole Exome Sequencing in Melanoma Revealed Sixteen Highly Mutated Genes

		Exome Capture (n=14)	Combined Exome Capture and Prevalence Screens (n=52)
Gene name	**P value**	**% of tumors affected**	**% of tumors affected**
BRAF	4.80E-05	50%	65%
GRIN2A	6.36E-03	43%	33%
CCDC63	3.34E-03	29%	11%
TMEM132B	7.59E-03	29%	17%
ZNF831	1.29E-02	29%	17%
PLCB4	4.39E-02	29%	15%
AKR1B10	5.21E-03	21%	8%
TAS2R60	5.46E-03	21%	9%
KHDRBS2	7.26E-03	21%	9%
PTPRO	9.09E-03	21%	8%
SYT4	1.23E-02	21%	8%
UGT2B10	2.13E-02	21%	8%
SLC6A11	2.84E-02	21%	6%
SLC17A5	7.91E-03	21%	6%
C12orf63	4.46E-02	21%	9%
PCDHB8	4.80E-02	21%	8%

Wei et al, *Nature Genetics*, 43:442-446 (2011)

GRIN2A is Highly Mutated in Melanoma (25%)



Recurrent mutations:

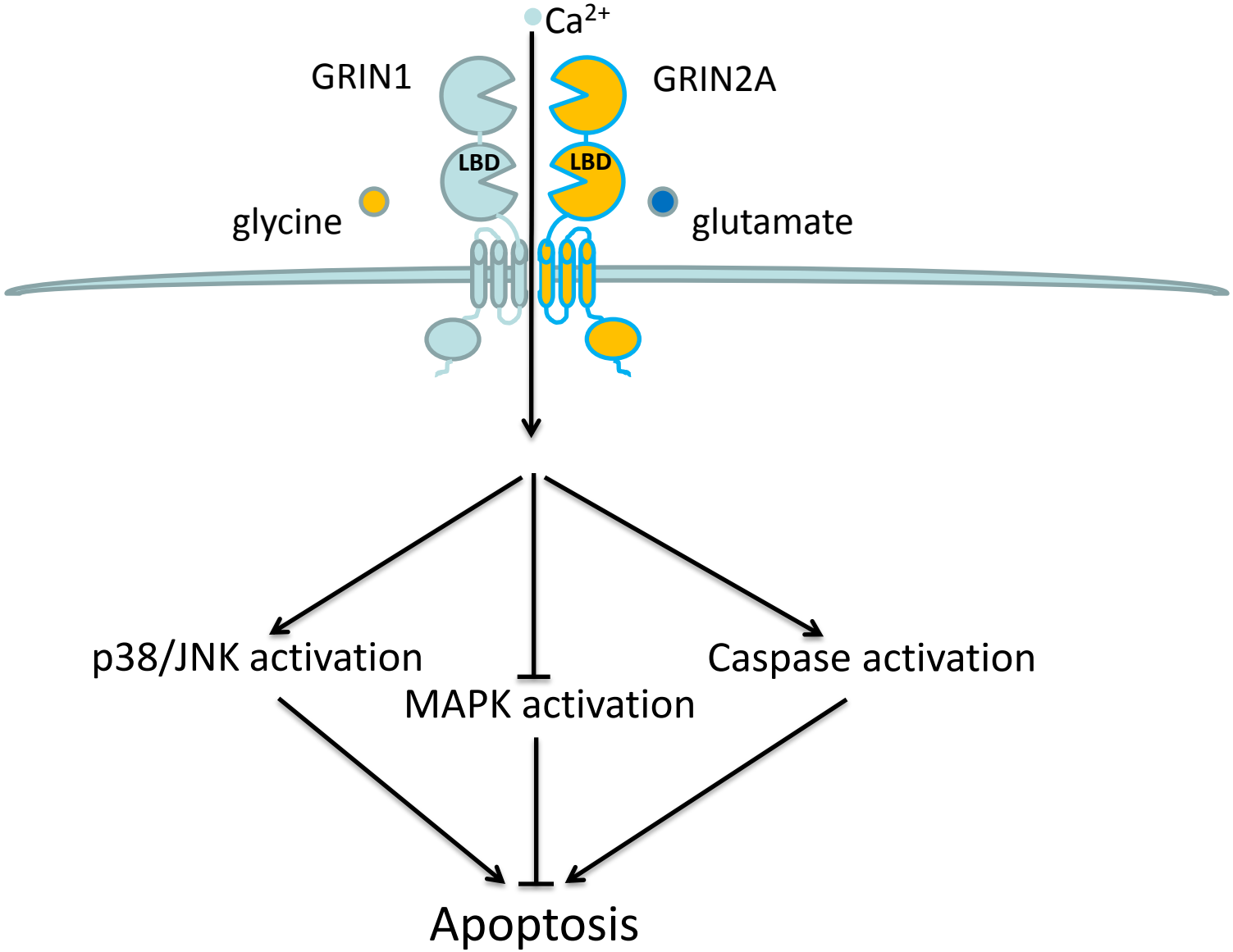
COSMIC

Garraway et al, *Nature (2012)* (4/25; 16%)

Chin et al , *Cell (2012)* (30/121; 25%)

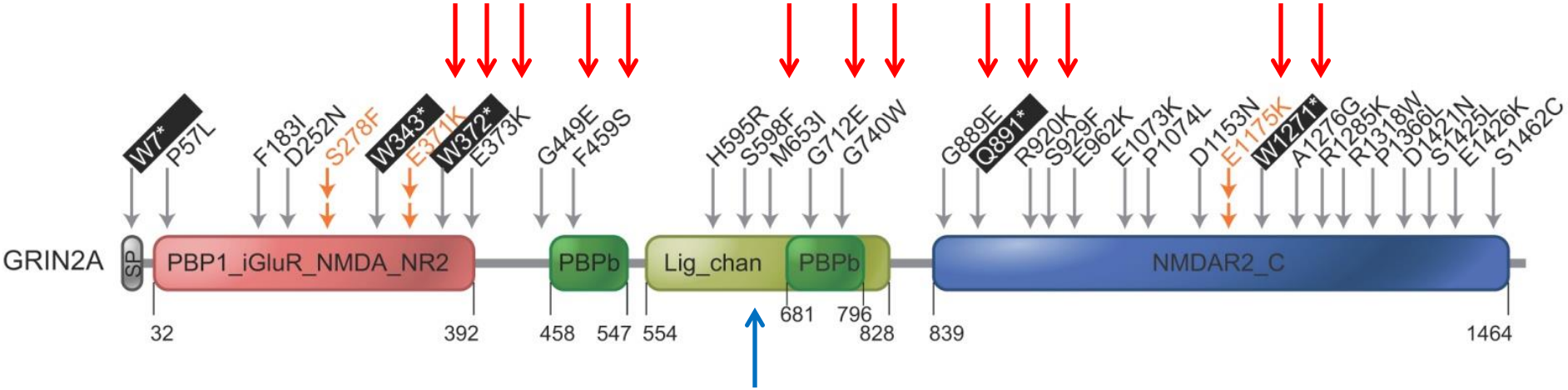
Wei et al, *Nature Genetics*, 43:442-446 (2011)

NMDA Receptor Structure and Function



LBD – ligand binding domain

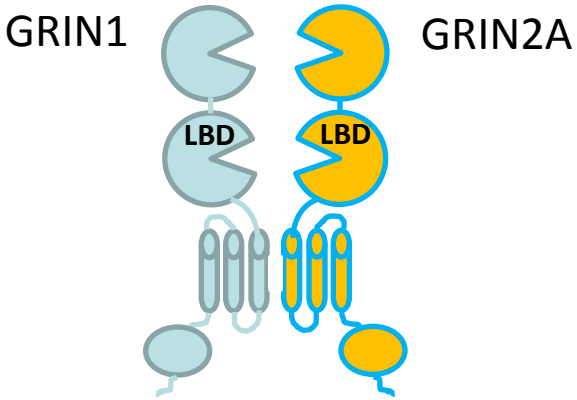
Cloned GRIN2A Somatic Mutations

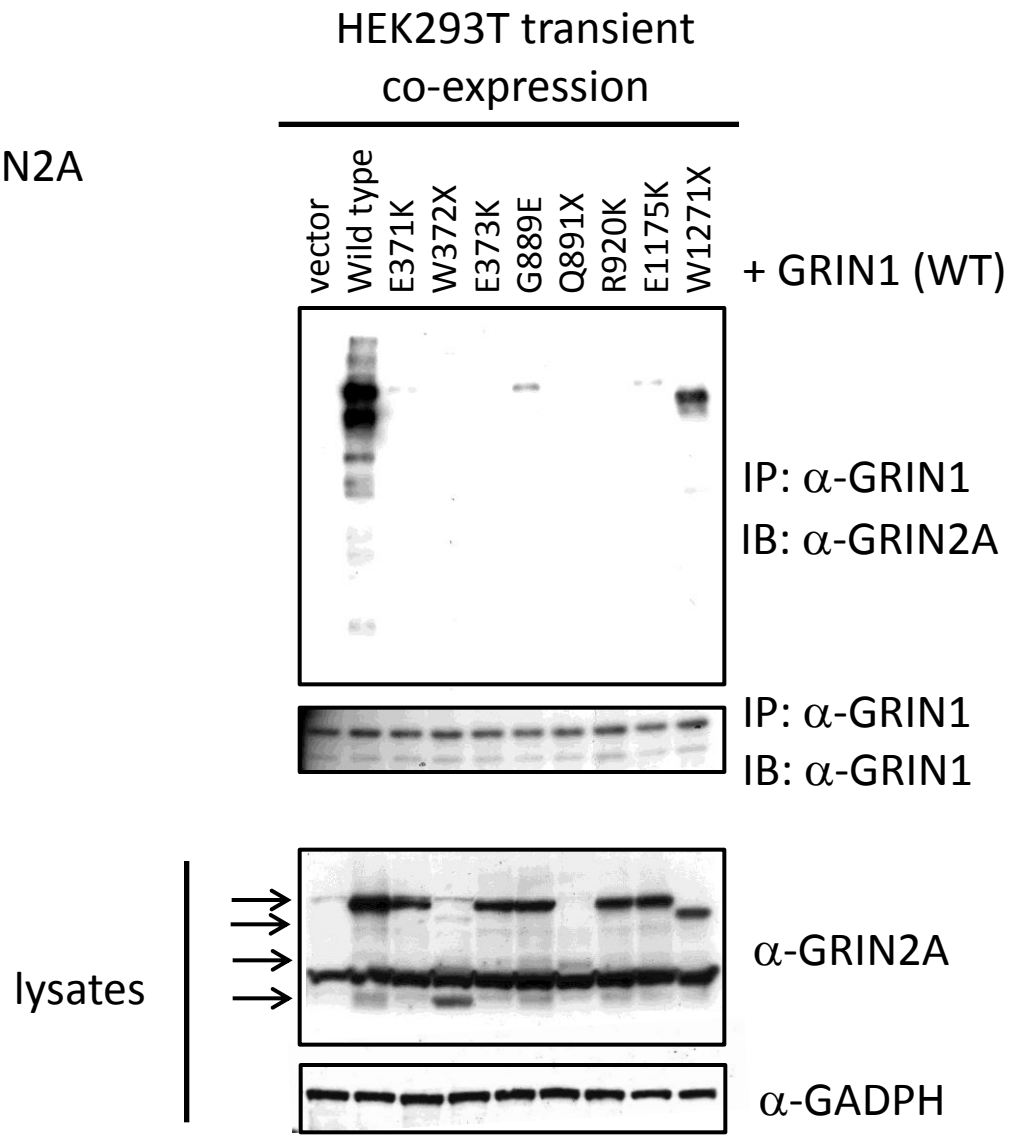


*NMDAR disrupting mutant
(Endele et. al., Nat Genet. 2010 (42) 1021-26)

Wei et al, *Nature Genetics*, 43:442-446 (2011)

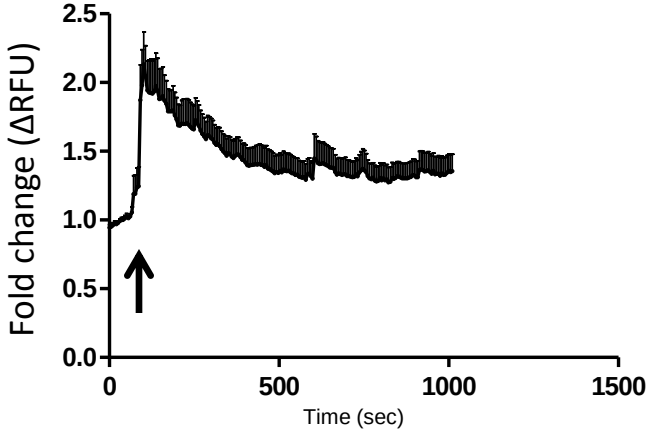
Somatic Mutations in GRIN2A Affect Complex Formation with GRIN1



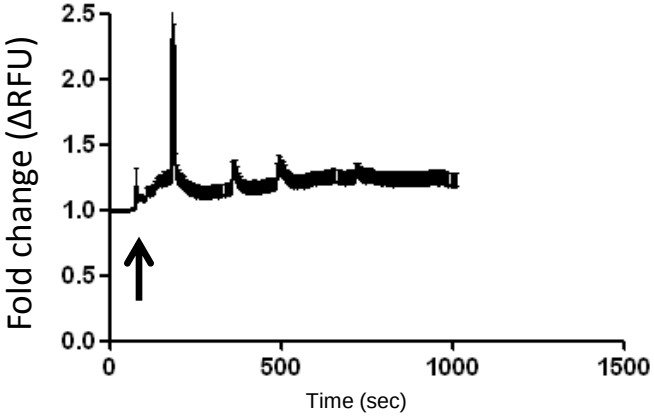


Mutant GRIN2A disrupts Ca^{2+} signaling

GRIN1 (WT)/
GRIN2A (WT)

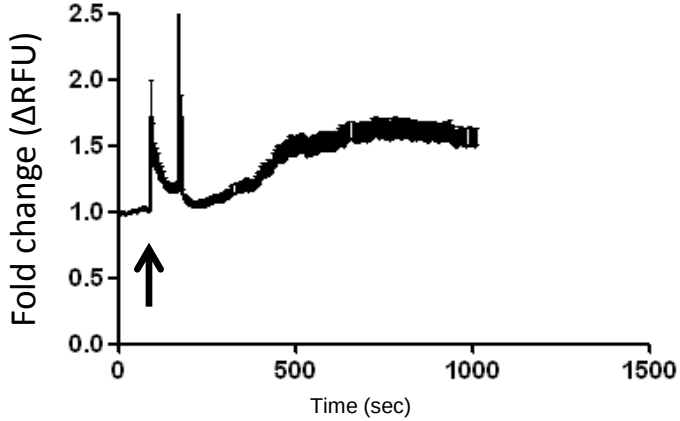


GRIN1 (WT)/
GRIN2A (Q891X)

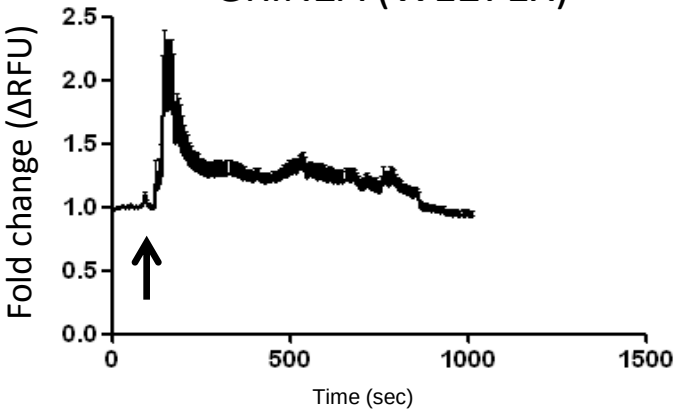


↑ 200μM NMDA added to cells

GRIN1 (WT)/
GRIN2A (R920K)

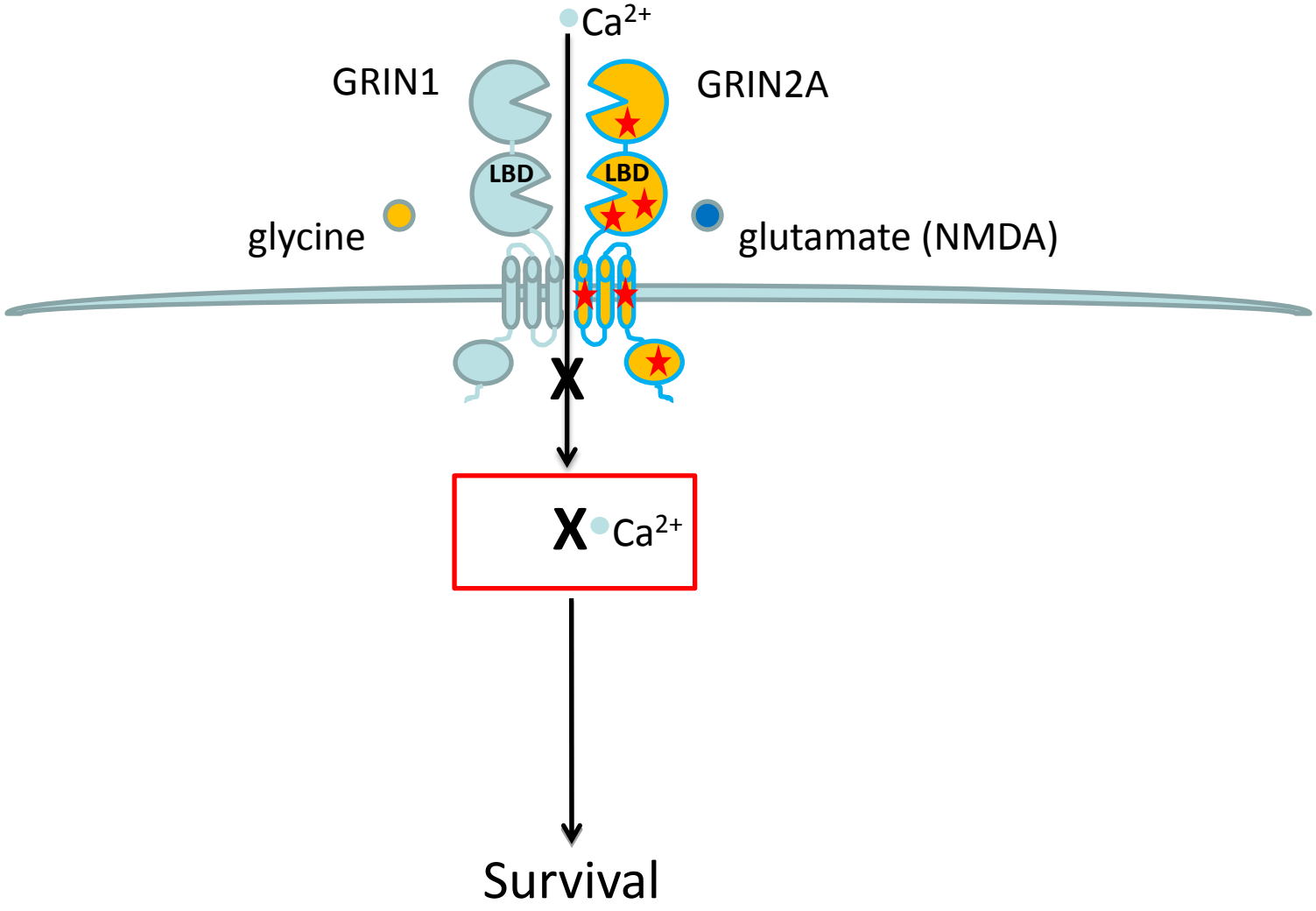


GRIN1 (WT)/
GRIN2A (W1271X)



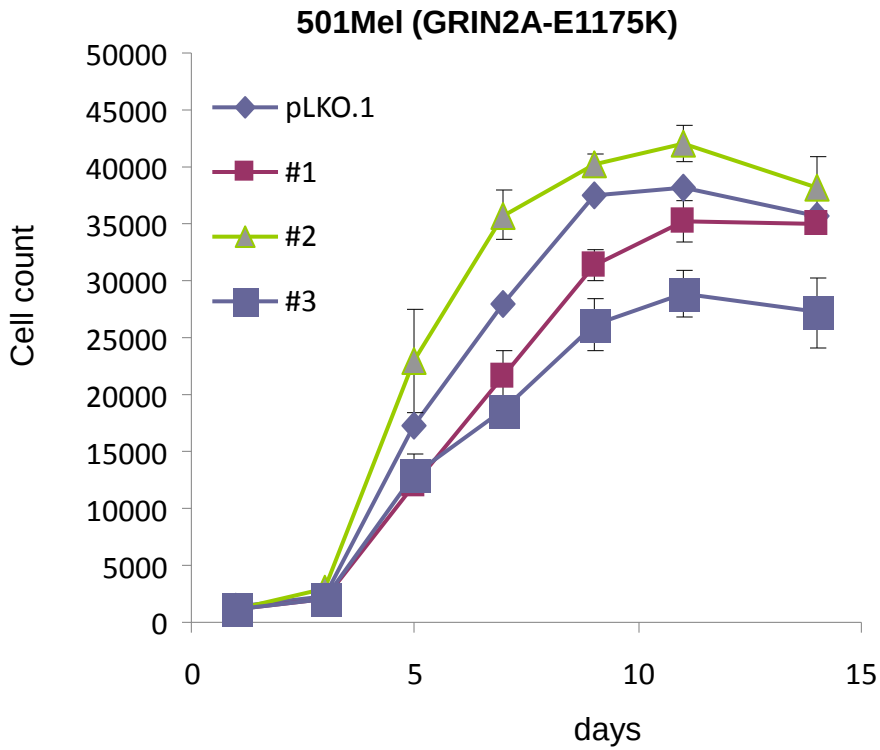
Silvio Gutkind

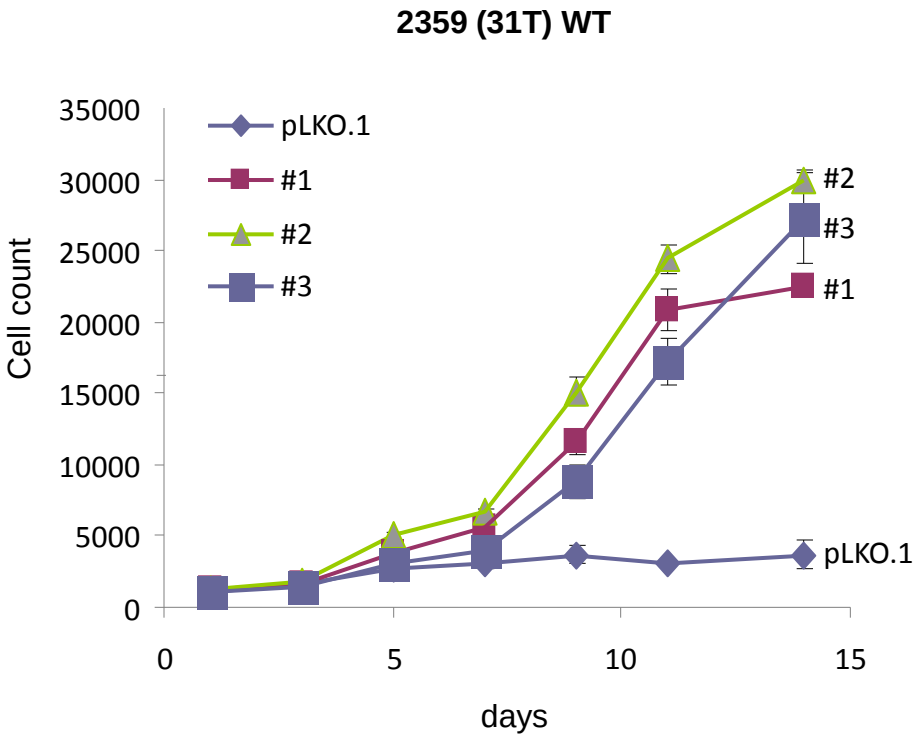
NMDA Receptor Structure and Function



LBD – ligand binding domain

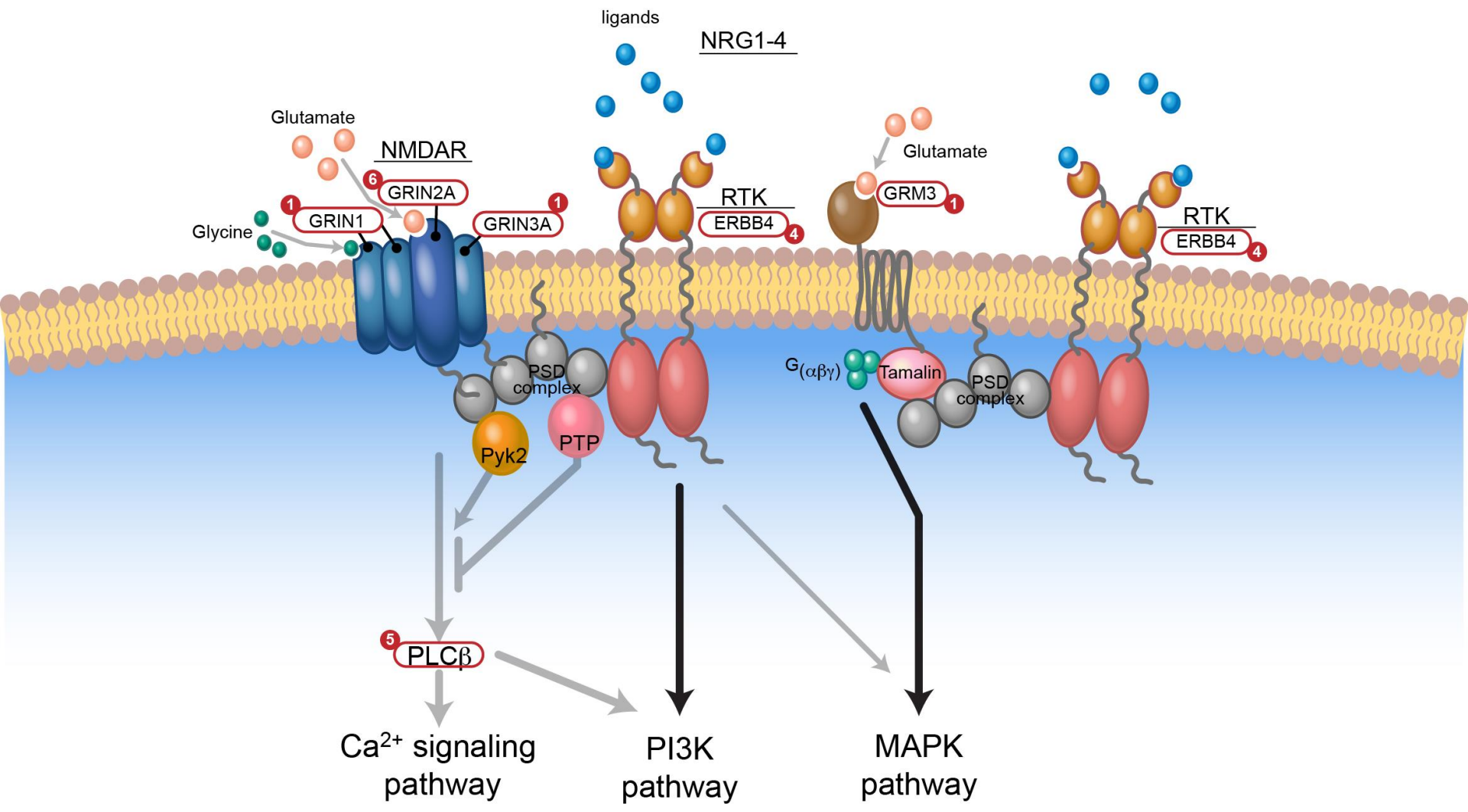
Expression of Wild-Type GRIN2A Inhibits Cell Proliferation





Pathway Oriented Models in Cancer Genetics

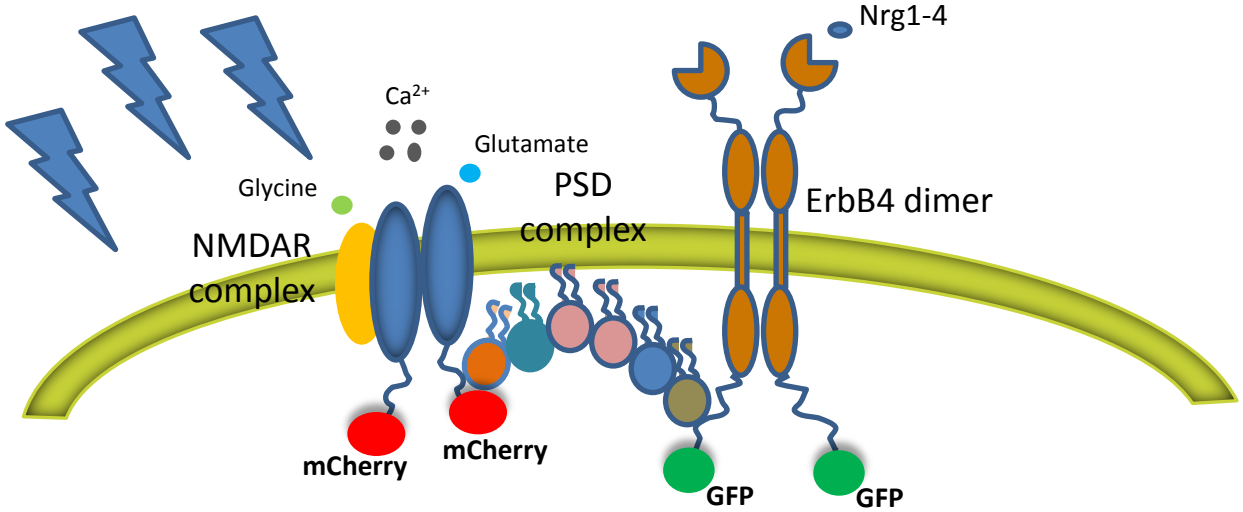
Mutations in the Glutamate Signaling Pathway are Enriched in Melanoma



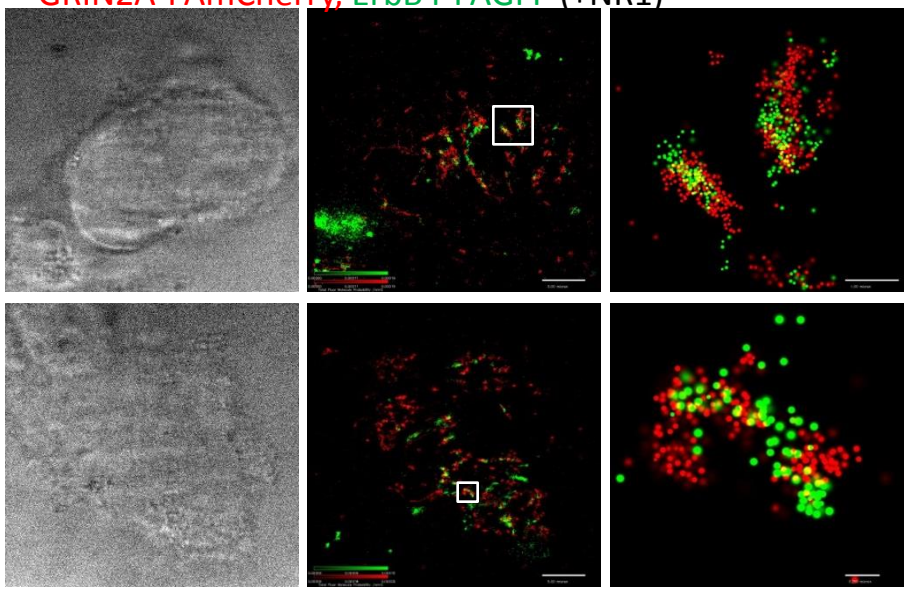
Binomial test-p-value 0.000237 (Gene Ontology, Kyoto Encyclopedia of Genes and Genomes, and MSigDB)

Photoactivated Localization Microscopy (PALM)
Imaging single molecules *in situ*

Photoactivate GFP and mCherry



GRIN2A-PAmCherry, ErbB4-PAGFP (+NR1)



A375 cells (10% serum)

Eilon Sherman

nature genetics

Exome sequencing identifies GRIN2A as frequently mutated in melanoma

Xiaomu Wei[1], Vijay Walia[1,12], Jimmy C Lin[2,12], Jamie K Teer[3], Todd D Prickett[1], Jared Gartner[1], Sean Davis[4],
NISC
Jeffre
lena Samuels[1]

nature genetics

letter

Melanoma mouse model implicates metabotropic glutamate signaling in melanocytic neoplasia

Pamela M. Pollock[1*], Karin
Hua Zhu[2], Christiane Robb
Laura M. Yudt[1], Amy Chen
Dunn[5], Steven M. Crespo-C
Kenneth R. Reuhl[4], Michael
*These authors contributed equally to

Expression of the metabotropic glutamate receptor 5 (mGluR5) induces melanoma in transgenic mice

Kyu Yeong Choi[a], Kai Chang[a], James M. Pickel[b], John D. Badger II[a], and Katherine W. Roche[a,1]
y, National Institute of Mental Health,

August 9, 2011 (received for review

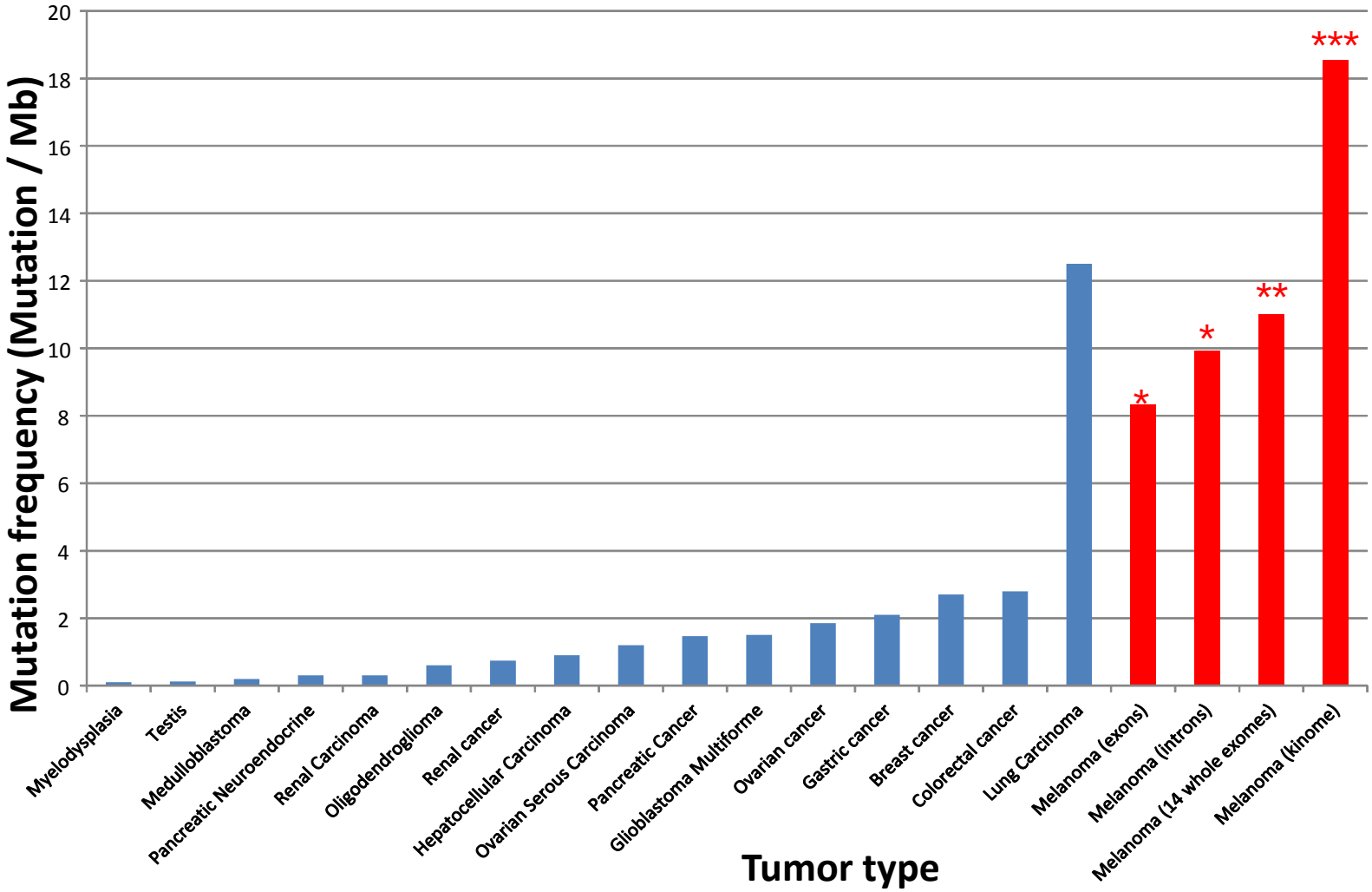
nature genetics

Exon capture analysis of G protein-coupled receptors identifies activating mutations in *GRM3* in melanoma

Todd D Prickett[1,11], Xiaomu Wei[1,11], Isabel Cardenas-Navia[1,11], Jamie K Teer[2,3], Jimmy C Lin[4], Vijay Walia[1], Jared Gartner[1], Jiji Jiang[1], Praveen F Cherukuri[2], Alfredo Molinolo[5], Michael A Davies[6,7], Jeffrey E Gershenwald[8,9], Katherine Stemke-Hale[7], Steven A Rosenberg[10], Elliott H Margulies[2] & Yardena Samuels[1]

Delving Deeper into the Melanoma Genome

Mutation Frequency in Solid Cancers



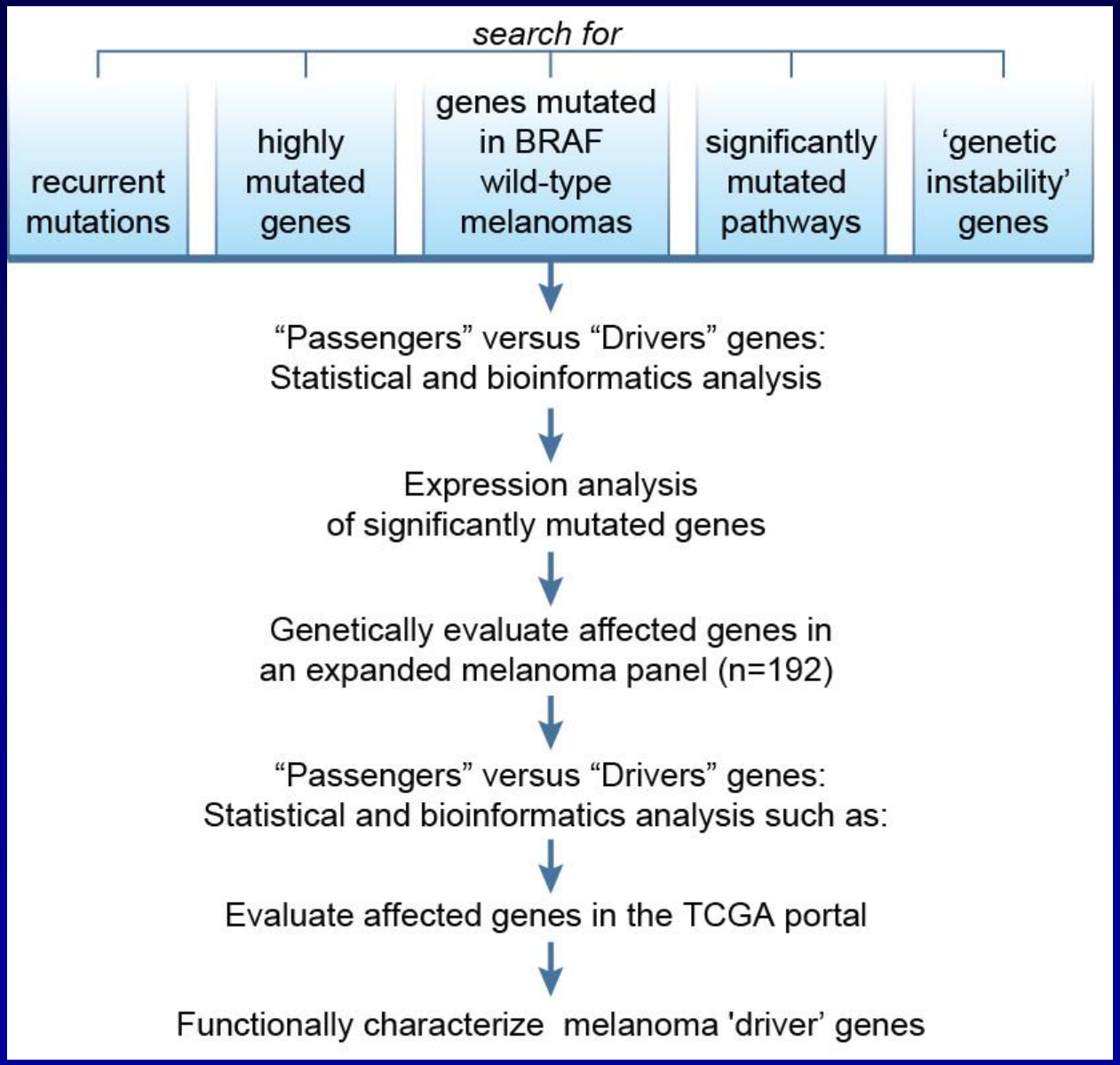
* Pleasance et al., Nature. 2010 Jan 14;463(7278):191-6

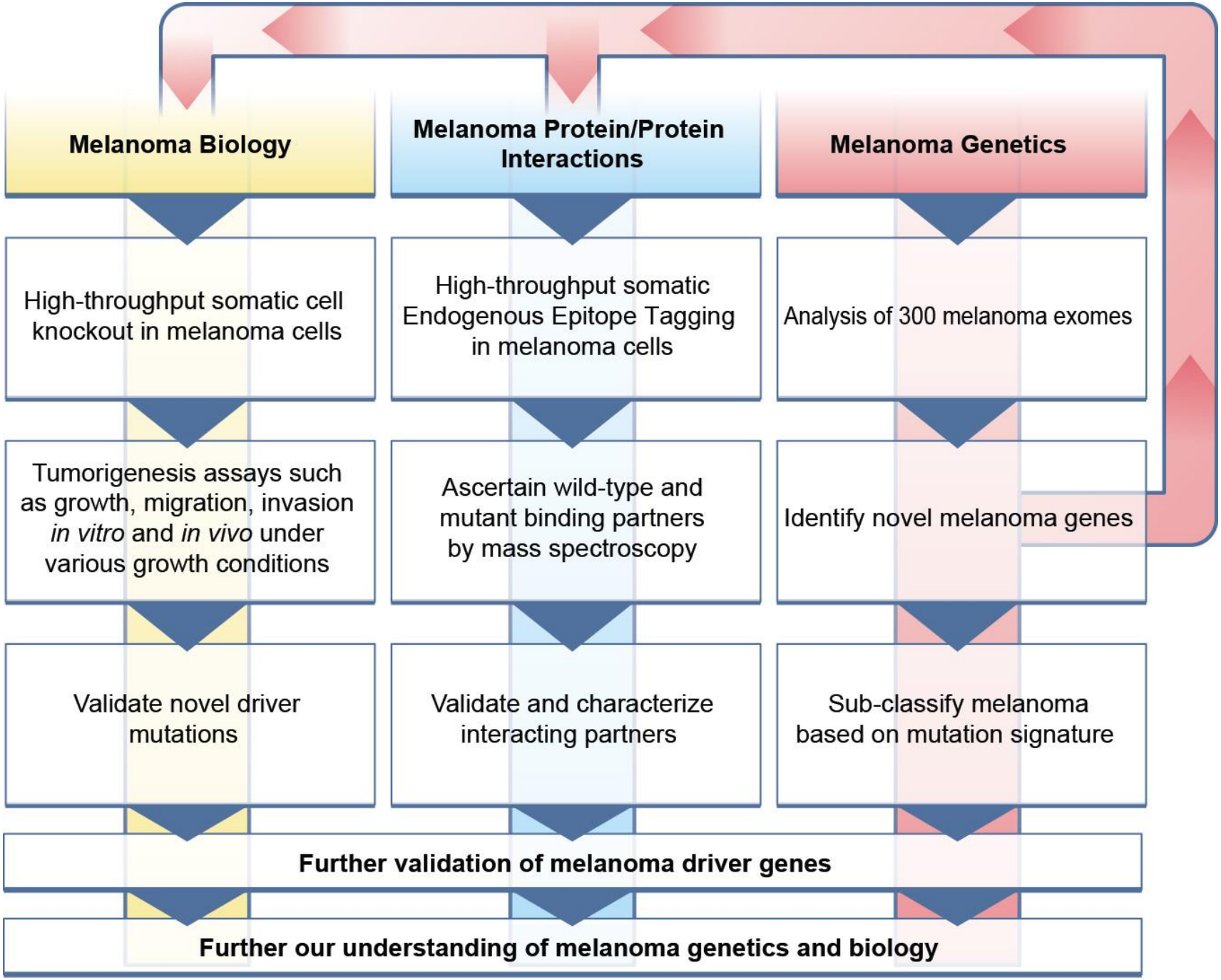
** Greenman et al., Nature. 2007 Mar 8;446(7132):153-8

*** Wei et al, *Nature Genetics*, 43:442-446 (2011)

Walia V et al, Pigment Cell Melanoma Res. 2012 Jan 19

Integration of Cutaneous Melanoma Somatic Mutation Data from 300 Whole Exomes/Whole Genomes




Melanoma Biology
Melanoma Protein/Protein Interactions
Melanoma Genetics
High-throughput somatic cell knockout in melanoma cells
High-throughput somatic Endogenous Epitope Tagging in melanoma cells
Analysis of 300 melanoma exomes
Tumorigenesis assays such as growth, migration, invasion *in vitro* and *in vivo* under various growth conditions
Ascertain wild-type and mutant binding partners by mass spectroscopy
Identify novel melanoma genes
Validate novel driver mutations
Validate and characterize interacting partners
Sub-classify melanoma based on mutation signature
Further validation of melanoma driver genes
Further our understanding of melanoma genetics and biology

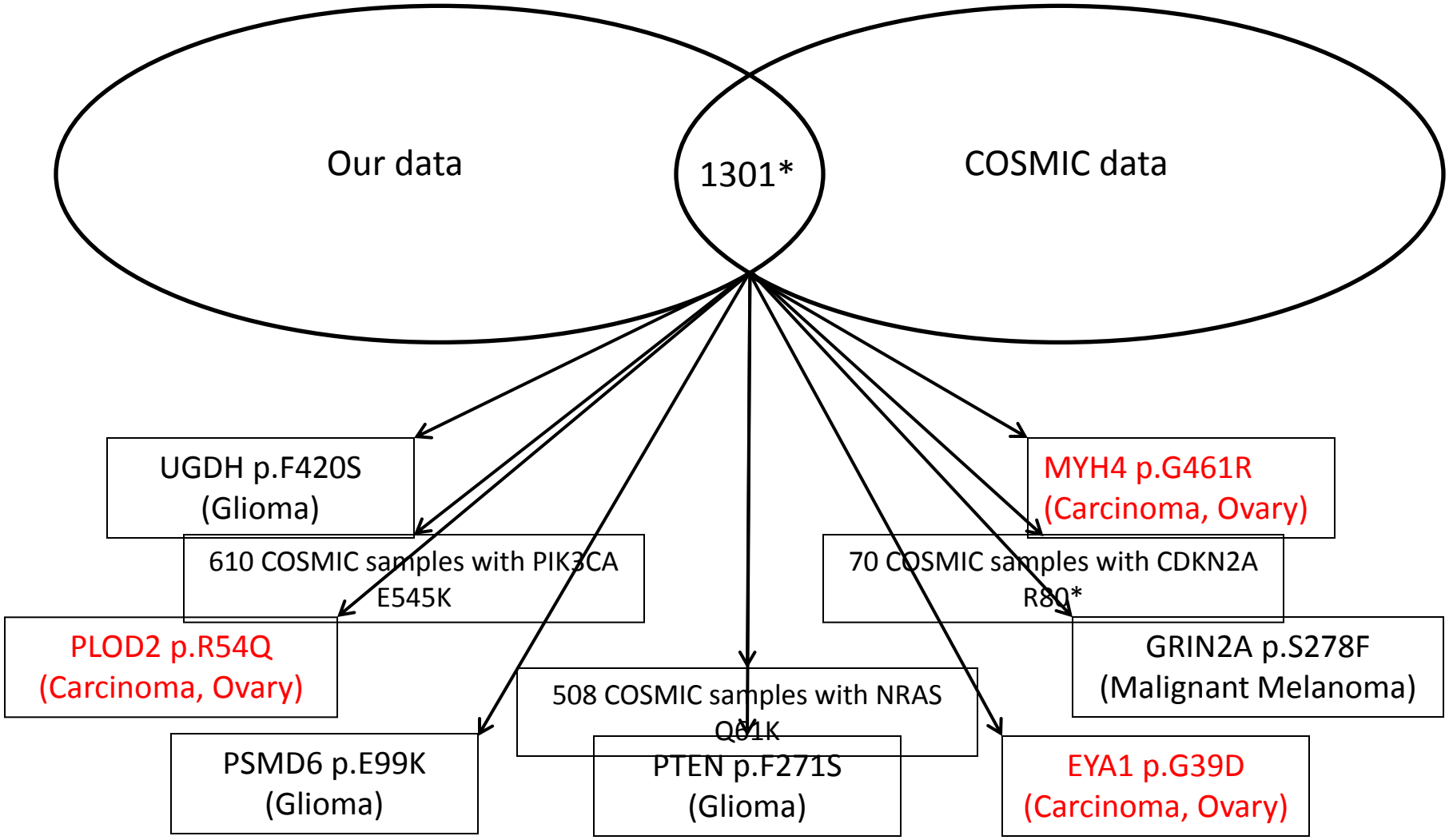
Integration Across Tumor Types-"Hotspots"

Merge of our data with COSMIC data revealed 8 non-synonymous "hotspot" mutations <u>shared</u> by 1301 samples*.

Known hotspot mutations (PIK3CA, NRAS, CDKN2A, EZH2, TP53, & BRAF) found in our samples.

Find 7 novel "hotspot" mutations*.

All found in one of our samples and one sample in COSMIC

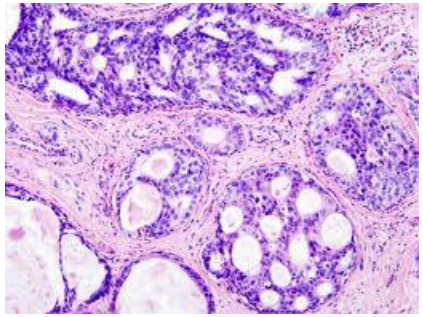


* this number excludes 11389 COSMIC samples containing the BRAF V600E

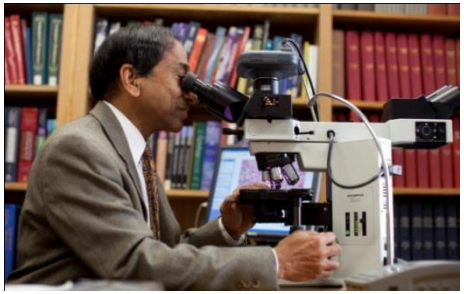
Mutations in red indicate found through whole genome screen

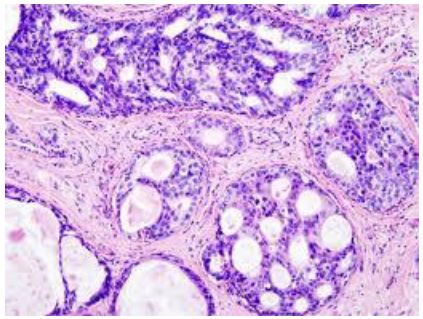
Genomic Medicine: Cancer Diagnostics

Now



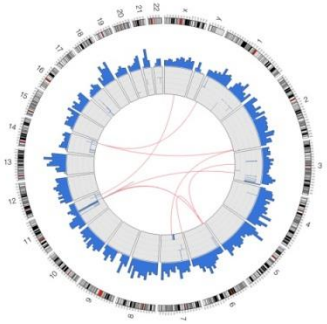
Future





+

©2011, Illumina Inc. All rights reserved.



Disease categorized by clinical, pathologic, and somatic mutation signatures

Courtesy Eric Green

Examples of Targeted Therapies In Cancer

Drug	Tumor type	Target gene
Imatinib (Gleevec)	CML	BCR/ABL
Trastuzumab (Herceptin)	Breast	HER2/NEU
EGFR inhibitors	Colorectal, Lung	EGFR
Vemurafenib	Melanoma	BRAF

Acknowledgments

Samuels Lab

Present

Todd Prickett

Victoria Hill

Brad Zerlanko

Jared Gartner

Past

Xiaomu Wei

Vijay Walia

Lavanya Palavalli

Jiji Jiang

Collaborators

Tumor Bank (NCI)

Steven Rosenberg

Paul Robbins

Nicholas Restifo

John Wunderlich

Udo Rudloff

Patti Fetch

Armando Filie

MD Anderson

Jeff Gershenwald

Mike Davies

Victor Prieto

Katherine Stemke-Hale

Colorado Medical Center

Steven Robinson

William Robinson

NIH

Elliott Margulies

Nancy Hansen

NISC

Jamie Teer

Steve Parker

Sean Davis

Nicholas Hayward (Queensland Institute of Medical Research)

Jimmy Lin (Washington University)

Klaus Elenius (University of Turku)

Eilon Sherman (Hebrew University)

New contact information

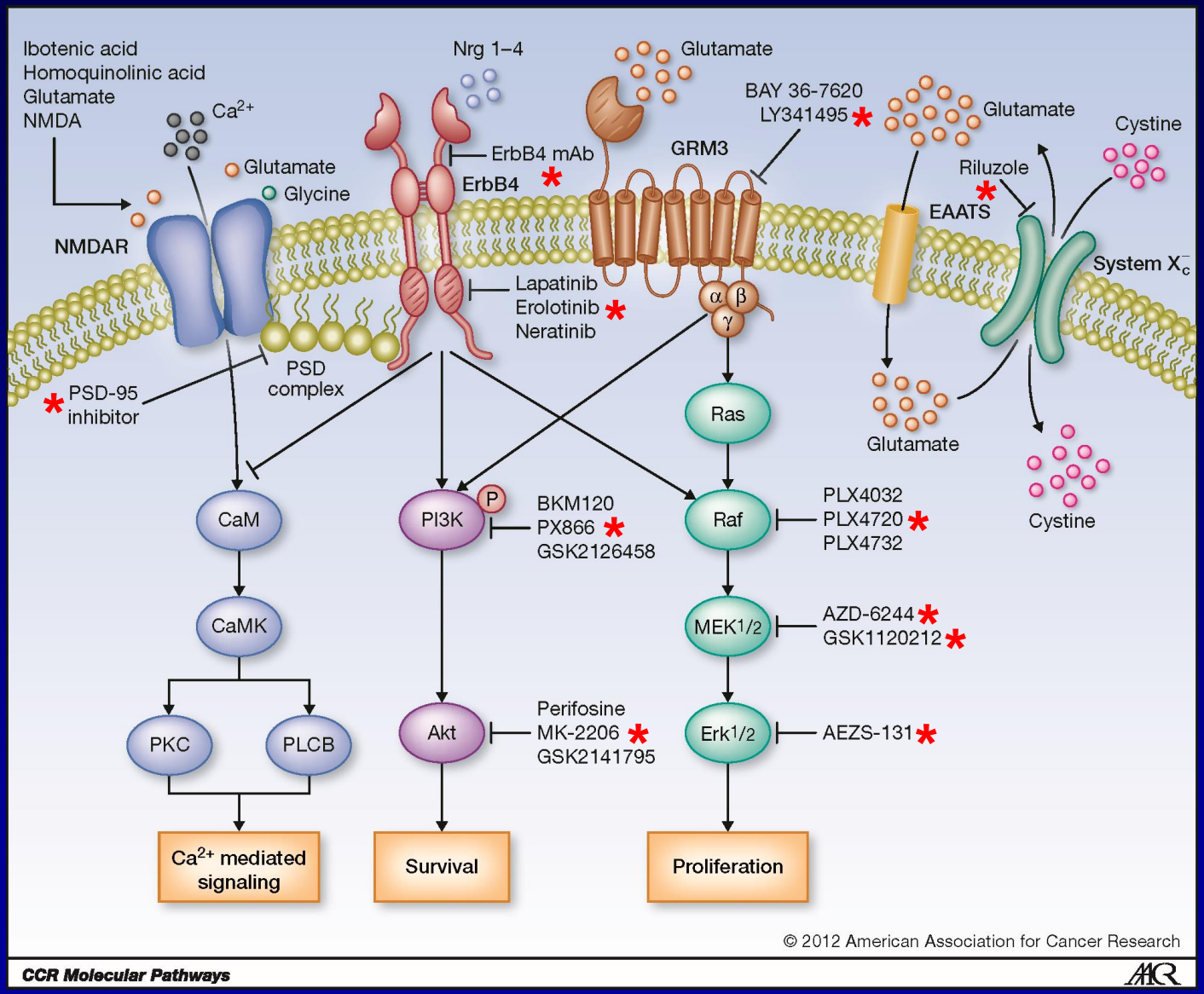
The Weizmann Institute of Science

Open positions:

Yardena Samuels, Ph.D.

Email: samuelsy@mail.nih.gov

Glutamate signaling pathways in melanoma



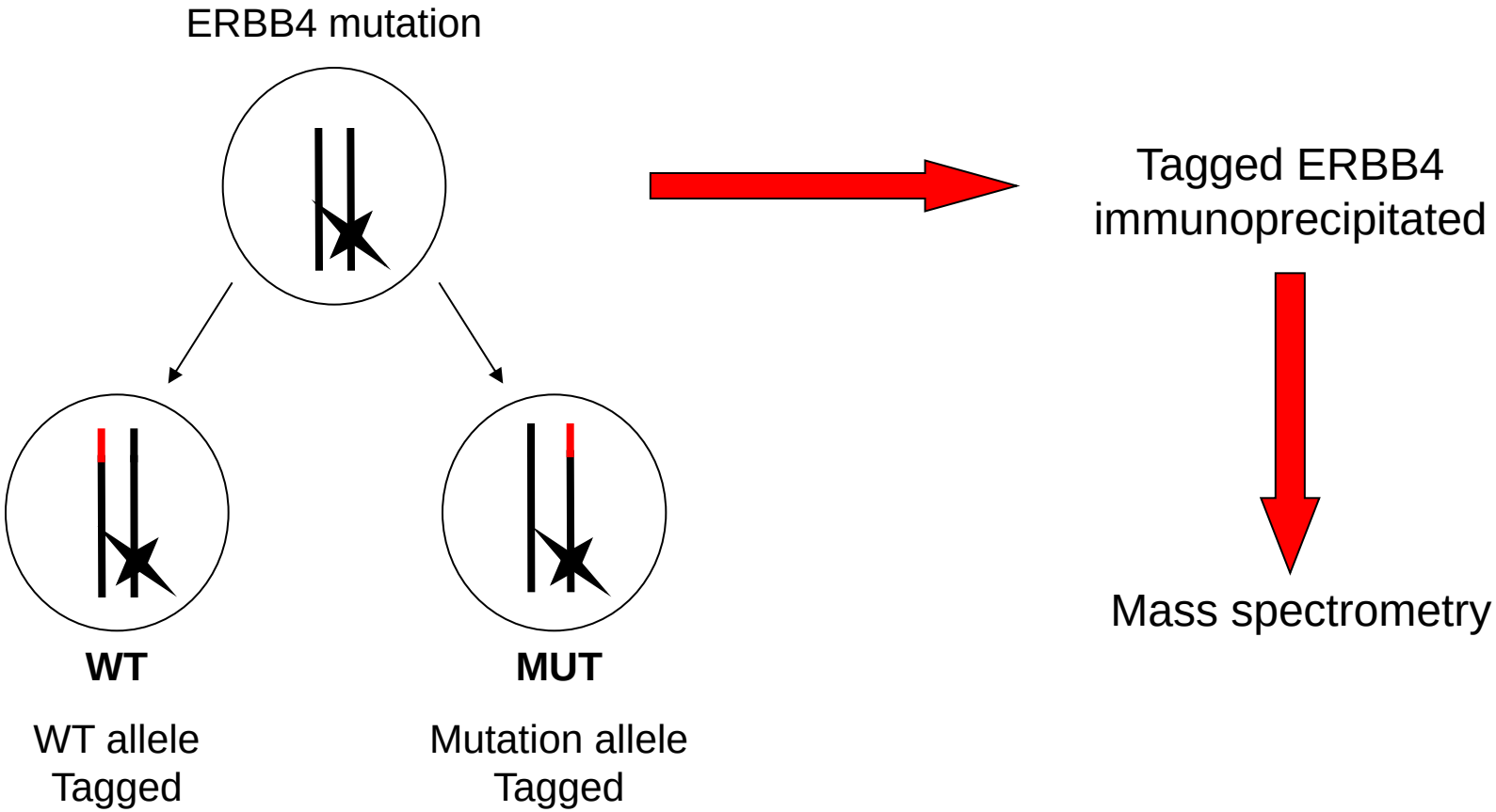
Prickett et al, *Clinical Cancer Research* (2012)

Identification of Melanoma Progression Genes

- Through the help of one of our collaborators we were able to obtain Primary, Metastasis, and Normal sample sets from 5 different patients.
- These samples are fresh DNA extracted from the sample at the time of removal.
- Whole exome sequencing of these samples may provide the opportunity to determine which variants are present in the metastasis and not the primary.
  - We could then evalutate these variants to see if/how they promote metastasis.

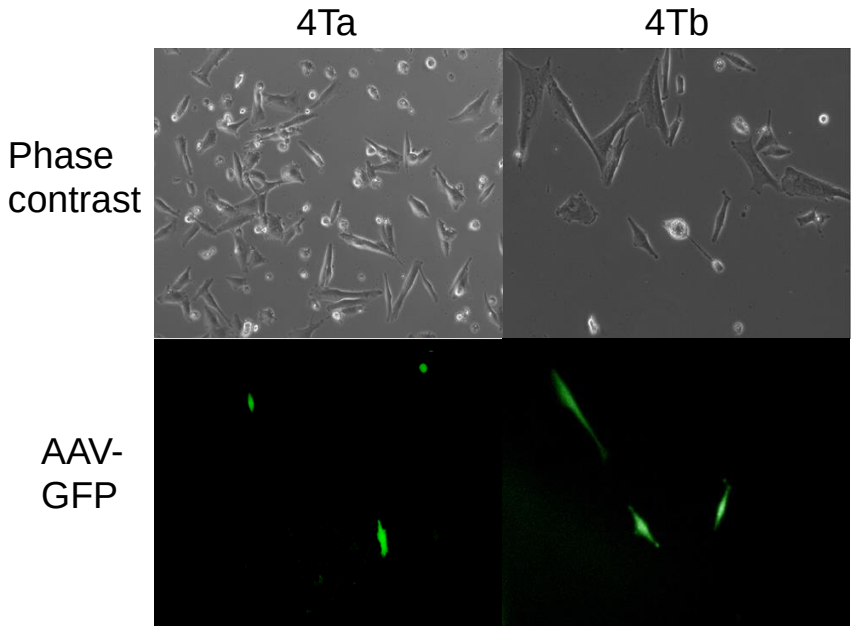
ERBB4 Interacting Proteins Strategy

The Endogenous Epitope Tagging (EET) Approach

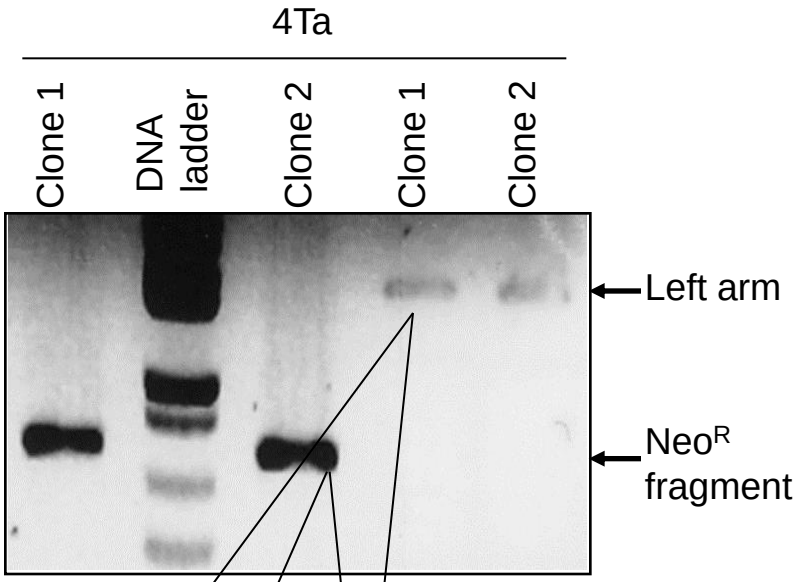


Flag Tag

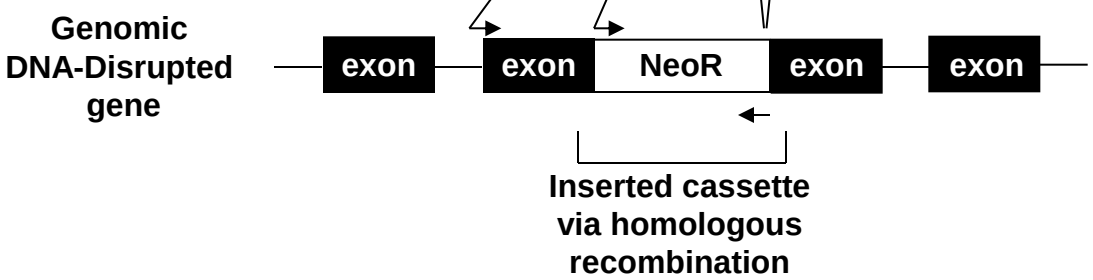
Preliminary Results



Melanoma cell lines can be infected by AAV



The rAAV construct can integrate into melanoma cell lines



Cutaneous Malignant Melanoma Overview

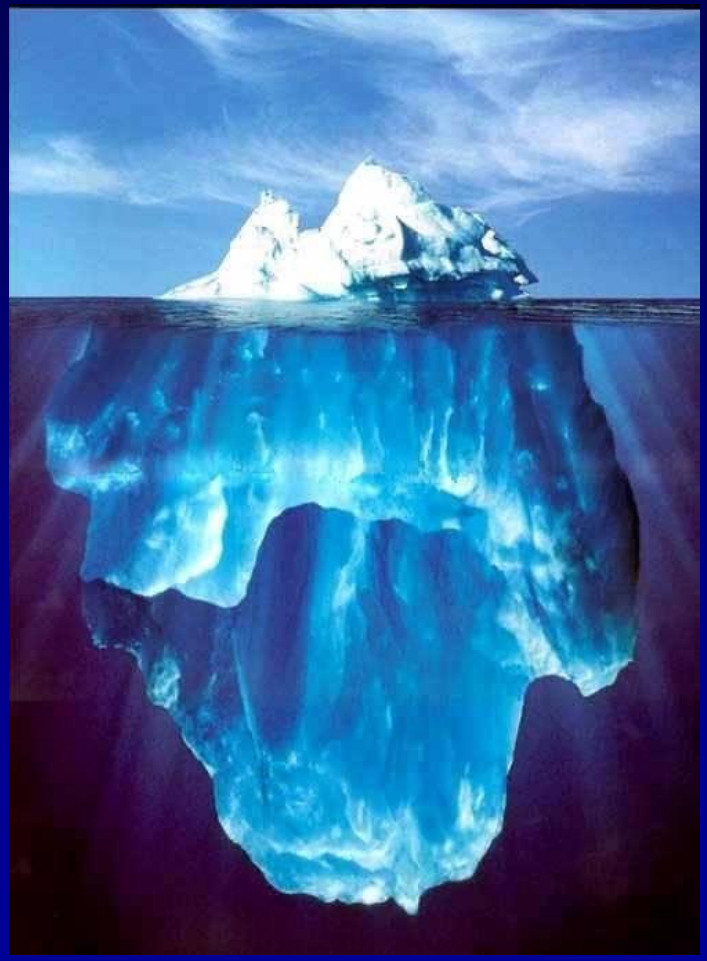
- 76,250 new cases of melanoma predicted (USA, 2012)
  9,180 people died of this disease predicted (USA, 2012)

- Median patient survival is six months following diagnosis of late stage disease

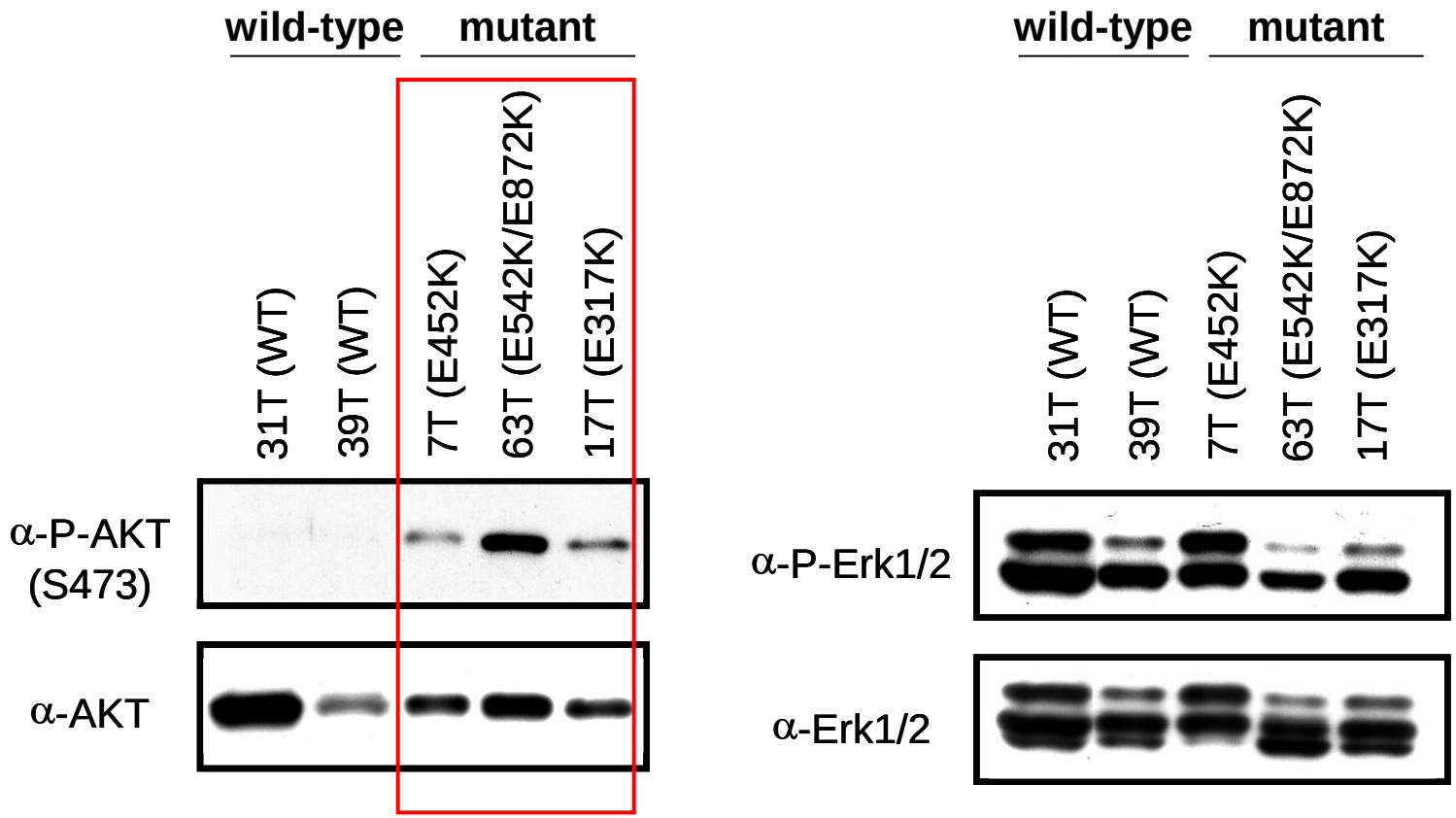
- Identification of genetic alterations may provide new opportunities for drug development

Seigel et al, CA Cancer J Clin. 2012 Jan;62(1):10-29.

What We Know About Cancer Genetics



Increased AKT Activation in Melanoma Cells Expressing Mutant ERBB4



Results of Initial Analysis

	Count of variants		
Sample Set name	Nonsynonymous (NS)	Synonymous (S)	NS + S
130 set	305	173	478
98 set	185	87	272
Totals	490	260	750

The G Protein Coupled Receptors Screen: Multiplex Capture

Discovery Phase
Location: NISC
No. of samples: 11
No. of genes: 734
Domain: 94% of coding exons

↓

Validation Phase
Location: Samuels lab
No. of samples: 80
No. of genes: 11
Domain: All coding exons

↓

Functional analysis

Somatic Mutations Identified in GRM3

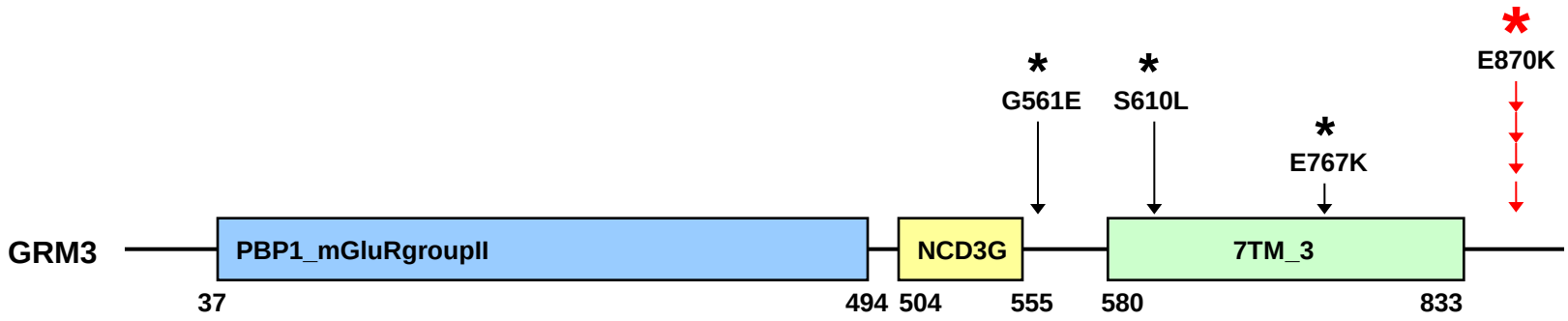
Cohort #1

Gene	Other names	CCDS accession*	Ref Seq accession*	No. of mutations (% tumors affected)#	Tumor	Exon	Nucleotide†	Amino Acid†	Functional domain‡
GRM3	GLUR3	5600.1	NM_000840	18 (16.3%)	85T	1	G53A	G18E	N/A
	GPRC1C				21T	1	G176A	R59Q	PBP1_mGluRgroupII
	MGLUR3				76T	1	G263A	G88E	PBP1_mGluRgroupII
	mGlu3				68T	1	C461T	S154F	PBP1_mGluRgroupII
					85T	2	C575T	P192L	PBP1_mGluRgroupII
					68T	2	G838A	D280N	PBP1_mGluRgroupII
					68T	2	C1054T	R352W	PBP1_mGluRgroupII
					29T	3	T/C (-13)	Splice Site	PBP1_mGluRgroupII
					6T	3	G1402T	V468L	PBP1_mGluRgroupII
					98T	3	G1531A	D511N	NCD3G
					39T	3	G1549A	E517K	NCD3G
					7T	3	G1642A	D548N	NCD3G
					36T	3	G1682A	G561E	N/A
					63T	3	G1717A	E573K	N/A
					76T	3	C1829T	S610L	7TM_3
					32T	3	G2299A	E767K	7TM_3
					22T	4	G2543A	G848E	N/A
					68T	5	G2608A	E870K	N/A

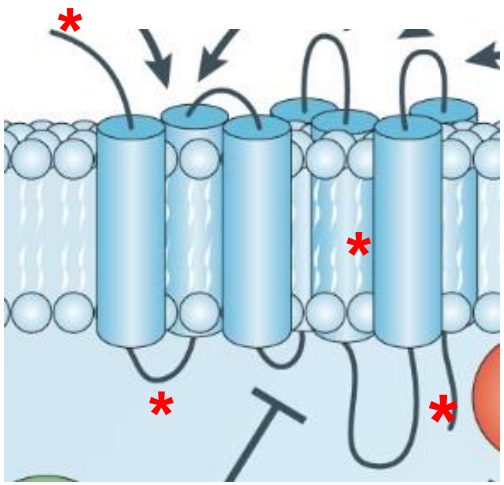
Cohort #2

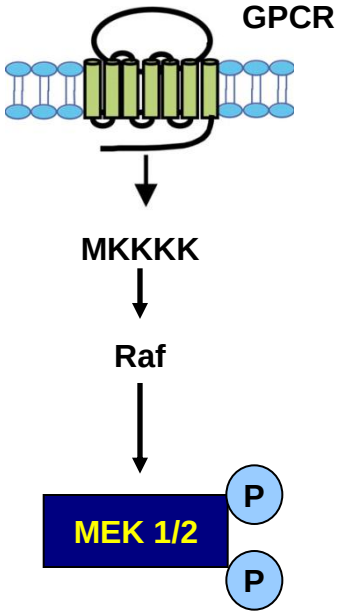
Gene	Other names	CCDS accession*	Ref Seq accession*	No. of mutations (% tumors affected)#	Tumor	Exon	Nucleotide†	Amino Acid†	Functional domain‡
GRM3	GLUR3	5600.1	NM_000840	11 (15.3%)	B05	exon 1	G289A	D97N	PBP1_mGluRgroupII
	GPRC1C				B05	exon 2	G538A	D180N	PBP1_mGluRgroupII
glutamate receptor, metabotropic 3	MGLUR3				B01	exon 2	G668A	G223E	PBP1_mGluRgroupII
	mGlu3				MD_22	exon 2	G682A	E228K	PBP1_mGluRgroupII
					B12	exon 2	G1065A	W355X	PBP1_mGluRgroupII
					A11	exon 3	T1640A	M547K	NCD3G
					MD_22	exon 3	T1640A	M547K	NCD3G
					MD_40	exon 3	T2009A	F670Y	7TM_3
					C19	exon 5	G2608A	E870K	N/A
					MD_05	exon 5	G2608A	E870K	N/A
					C17	exon 5	G2608A	E870K	N/A

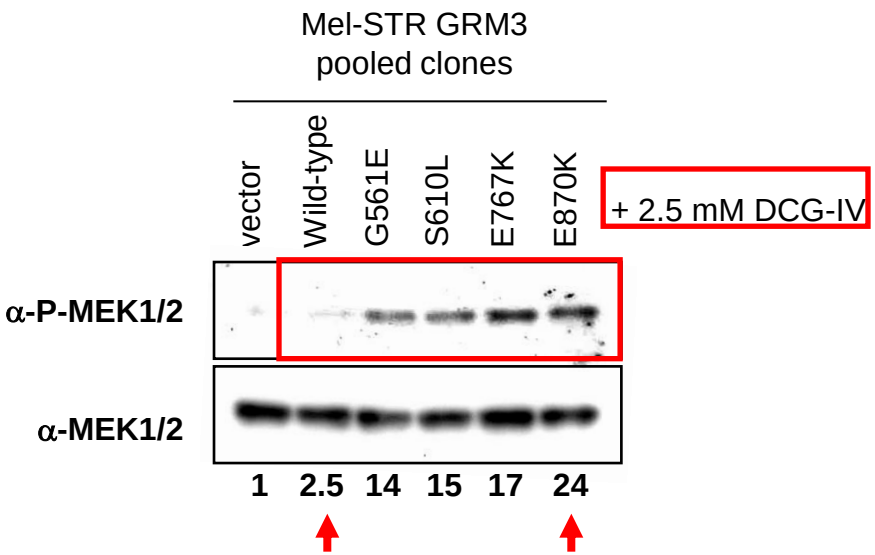
GRM3 Somatic Mutations Activate the MEK Pathway



The likelihood of having 4 identical mutations at the same position in 140 samples is 1.8×10^{-12}







Adapted from Wheeler C. et al, *Heart 2005;91:1366-1374*

Prickett et al, *Nature Genetics*, 43:1119-1126 (2011)

Whole Exome

Exome sequencing of **18** untreated melanoma samples and their matched normals. 180X coverage, 90% bases with high genotype call.

Whole Genome

Genome sequencing of **10** untreated melanoma samples and their matched normals. 41X mean coverage, 95% mean callable genome.

Merge data

Search for highly mutated genes

Search for non-synonymous recurrent mutations

38 recurrent non-synonymous mutations

15 genes found to be highly mutated

4 genes previously identified to be mutated and play a role in melanoma

Gene name	P-value	% tumors affected	Reference
BRAF	3.30E-20	63.64	Davies, H. et al. *Nature* 417, 949-54 (2002)
ERBB4	2.72E-07	18.18	Prickett, T.D. et al. *Nat Genet* (2011)
ADAM29	2.32E-10	18.18	Wei, X. et al. *Hum Mutat* 32, E2148-75 (2011)
GRM3	1.23E-04	18.18	Prickett, T.D. et al. *Nat Genet* (2011)

Elliott Margulies

Integration of melanoma somatic mutation data from 300 whole exomes/whole genomes

Search for **recurrent** mutations

Search for **highly** mutated genes

Search for genes mutated in **BRAF wild-type** melanomas

Search for significantly mutated **pathways**

Search for **'genetic instability'** genes

↓

"Passengers" versus "Drivers" genes:
Statistics and Bioinformatic analyses

↓

Expression analysis
of significantly mutated genes

↓

Genetically evaluate affected genes in
an expanded melanoma panel (n=192)

↓

"Passengers" versus "Drivers" genes:
Statistics and Bioinformatic analyses

↓

Evaluate affected genes in the TCGA portal for:
Somatic mutations; expression; copy number variations and methylation

↓

Functionally characterize
melanoma 'driver' genes

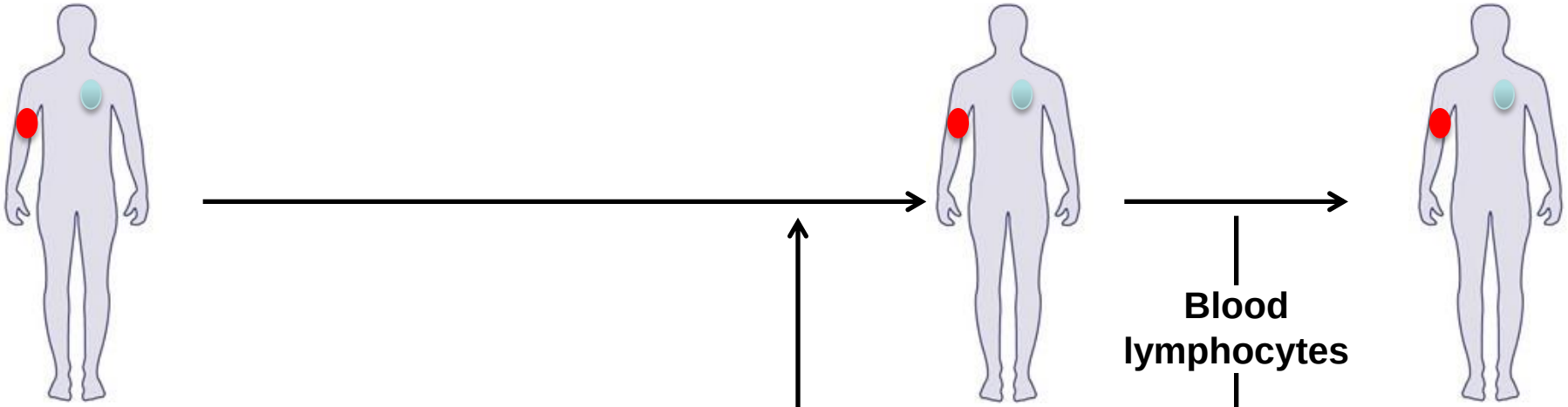
Criteria for Evalution

- Criteria was the same as used in our tumor normal whole exome sequencing.
  - MPG score of >10 MPG
  - MPG/Coverage of >0.5
  - For any variant this criteria needed to met in both metastases and the normal.

Gartner et al, *BMC Genomics*, Accepted (2012)

Mutation Specific Immunotherapy for Melanoma

Tumors are infiltrated by Tumor Infiltrating Lymphocytes (TILs) which may control malignant growth



-Whole exome sequencing
-HLA typing
-Predict mutated epitopes
-Predict best HLA binders

Adoptive transfer of autologous TILs stimulated *in vitro*

Assay for specific TIL responses

Clinical Benefit?

Retrospective study
N=8
Long term survivors
Positive results: PPP1R3B, GAS7

In collaboration
Steven Rosenberg
Paul Robbins

The Kinome Screen

Discovery Phase
Location: NISC
No. of samples: 29
No. of genes: 86
Domain: Kinase

↓

Analysis:
Mutation validation
Somatic mutation validation

↓

Validation Phase
Location: Samuels lab
No. of samples: 80
No. of genes: 19
Domain: All coding exons

↓

Analysis:
Mutation validation
Somatic mutation validation

↓

Functional analysis

Whole Exome Sequencing

Discovery

Exome capture (14 tumors/ matched normal)
Agilent SureSelect 37Mb
~20,000 genes and flanking regions
Illumina GAII platform
ELAND followed by cross_match

Validation

Sanger

- **2.4% false negative rate**
- **81% sensitivity**

Wei et al, *Nature Genetics*, 43:442-446 (2011)