New insights into melanoma pathobiology

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Disclosure slide

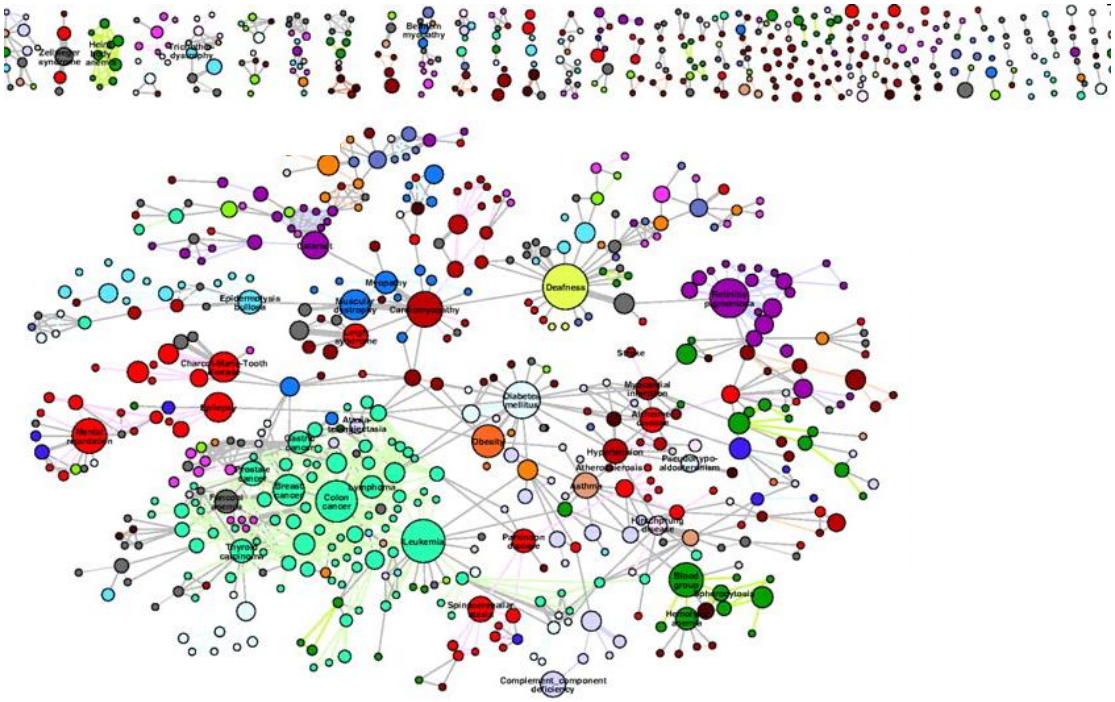
- I have no Conflicts of Interest to declare

The Road to Progress: From Intuition to Precision Medicine

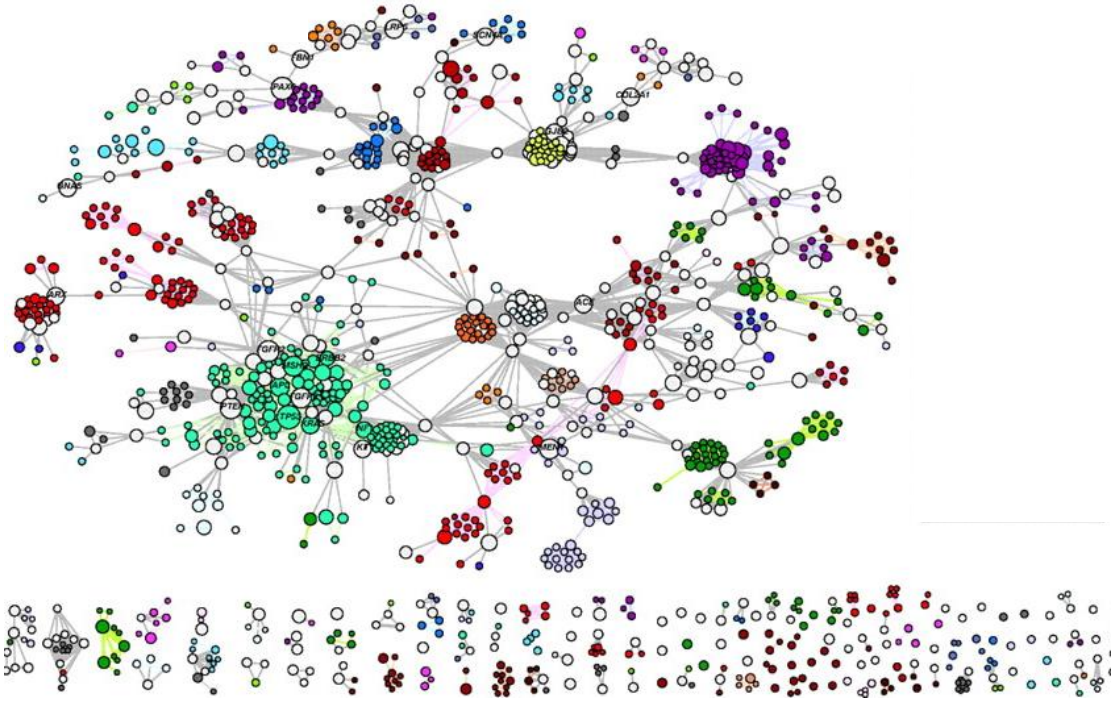
- **Intuitive Medicine:** Highly trained (and expensive) specialist solve medical problems through intuitive experimentation and pattern recognition
- **Empirical Medicine:** Evidence-based (data-driven) approaches demonstrated to, on average, perform better than alternatives
- **Precision Medicine:** Mechanism-based diagnosis and treatment with highly reproducible results and predictable outcomes

C. Christensen

Diseases



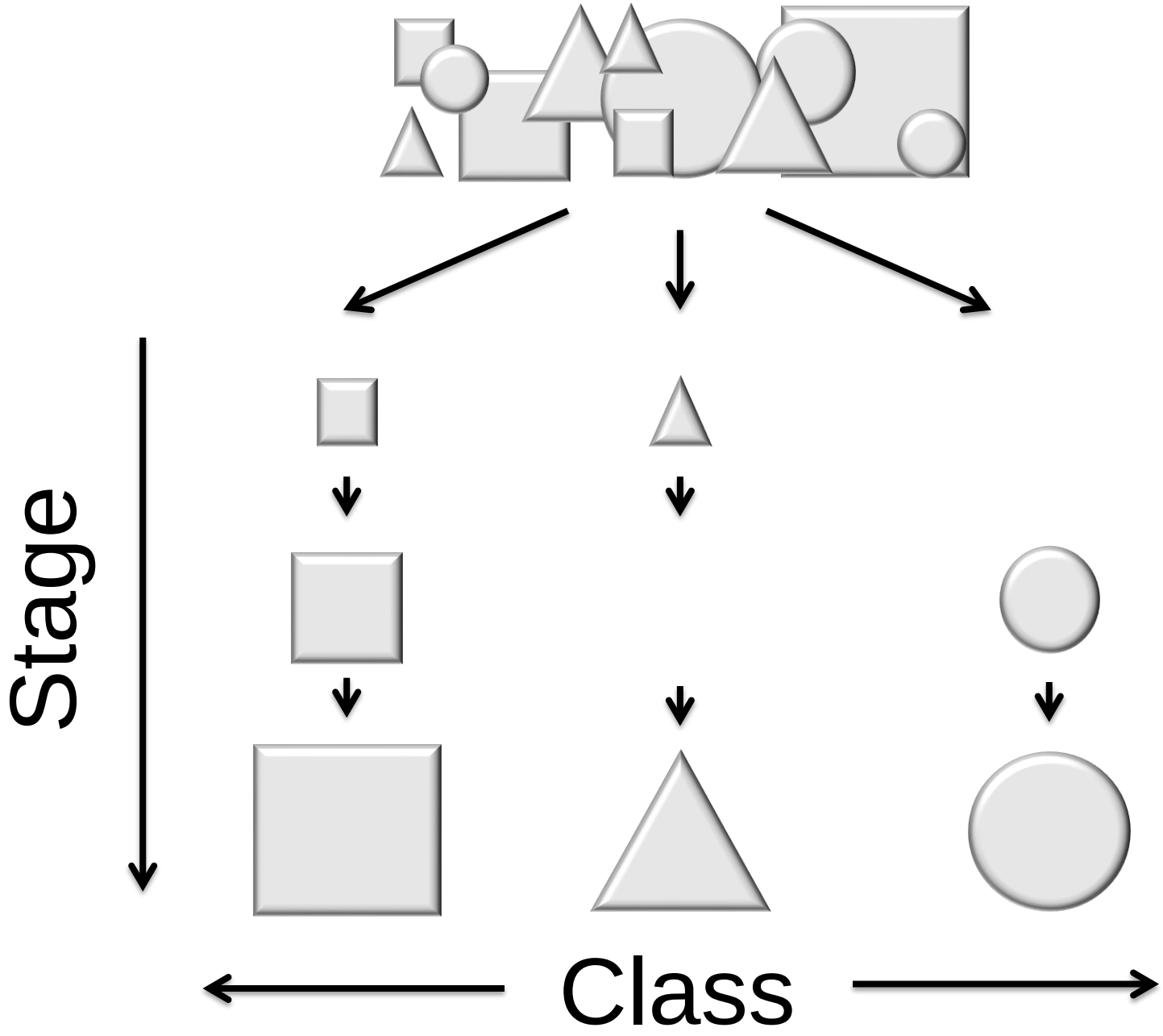
Mechanisms



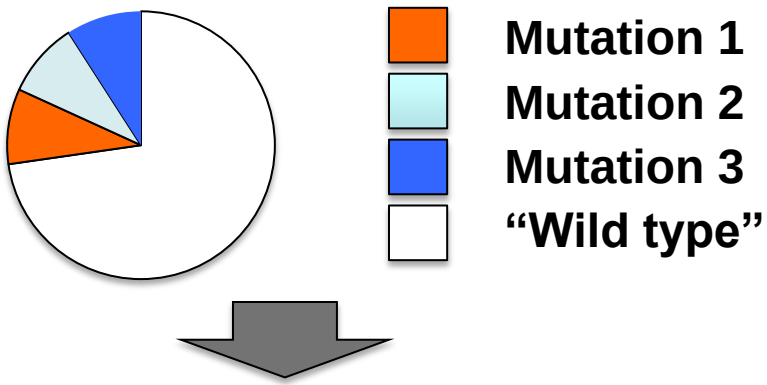
WHO Classification of Melanoma

- Superficial spreading melanoma
- Nodular melanoma
- Lentigo maligna melanoma
- Desmoplastic melanoma
- Nevoid melanoma
- Acral-lentiginous melanoma
- Mucosal melanoma
- Uveal melanoma
- Melanoma of childhood
- Melanoma arising from giant congenital nevus
- Melanoma arising from a blue nevus
- Persistent melanoma

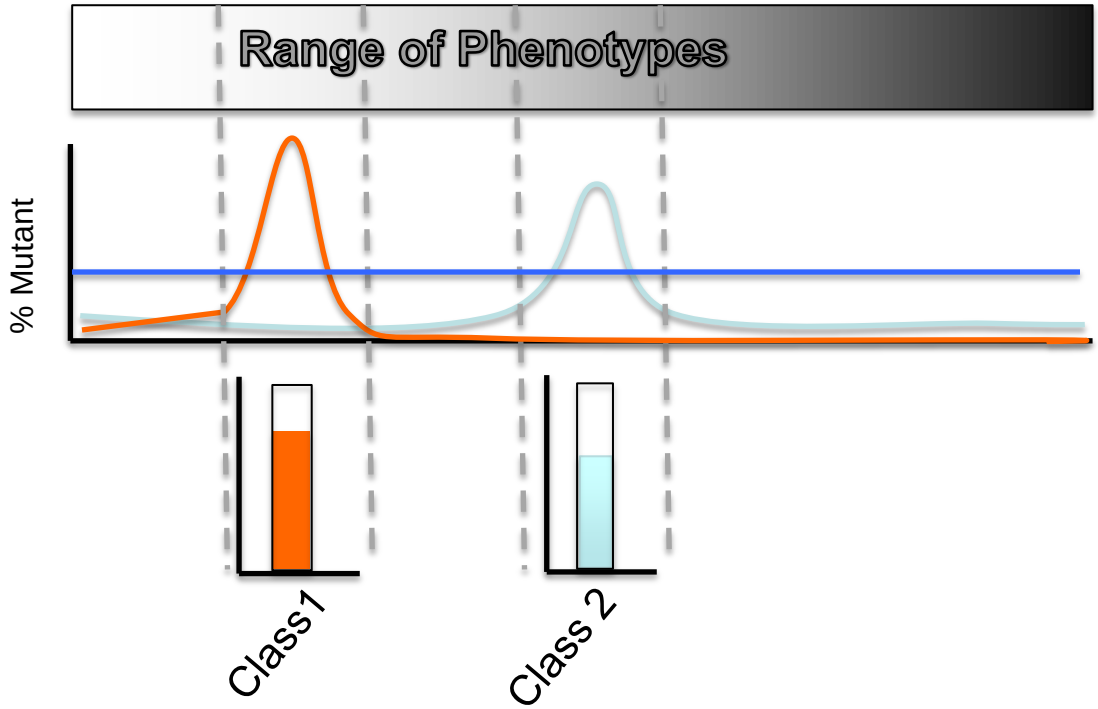
Clinically revelant disease classification have to use mechanism as the guiding principle


Stage
Class

Mutation frequency in all melanomas

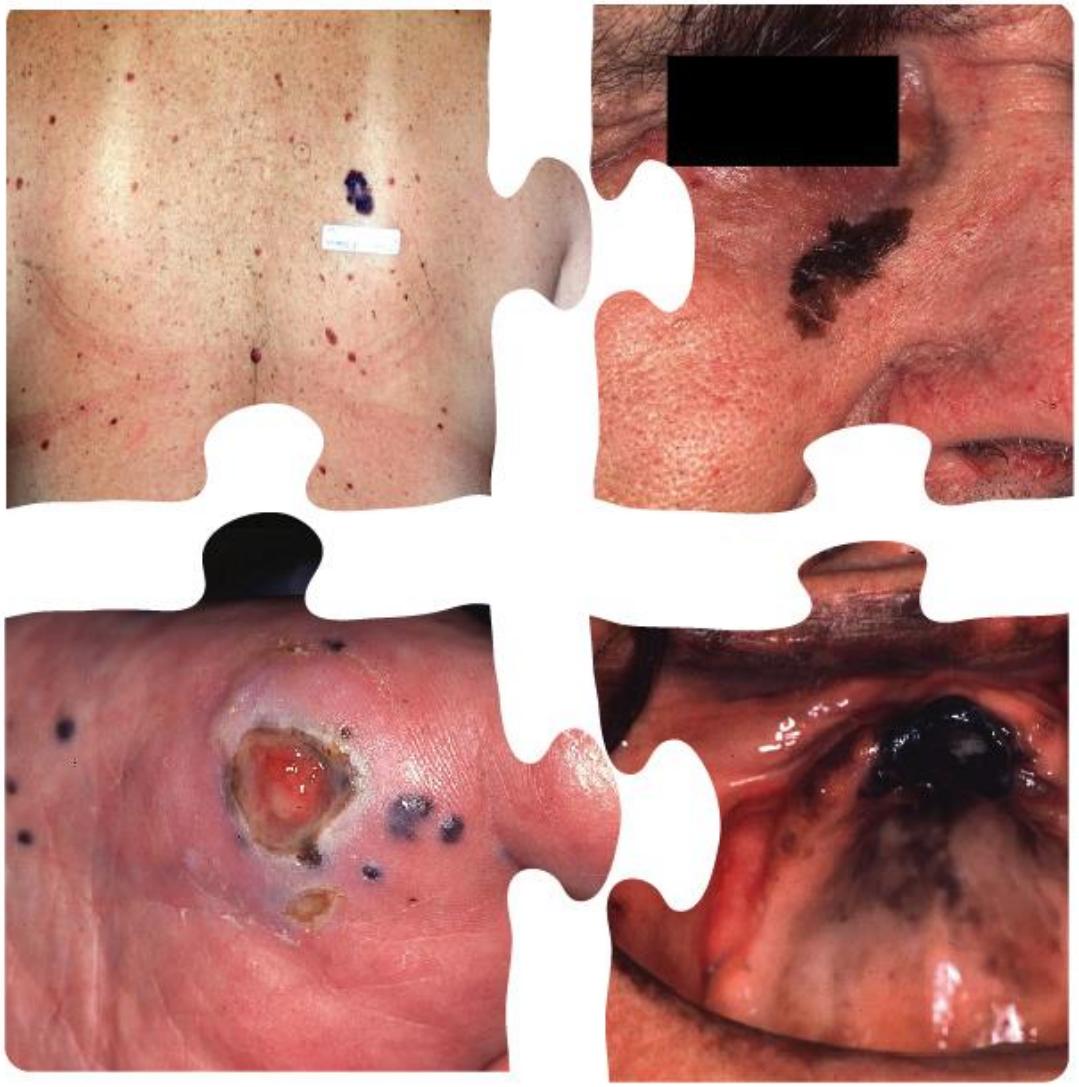


Mutation frequency across phenotypes



Genotype – Phenotype Correlations in Primary Melanomas

BRAF, NRAS



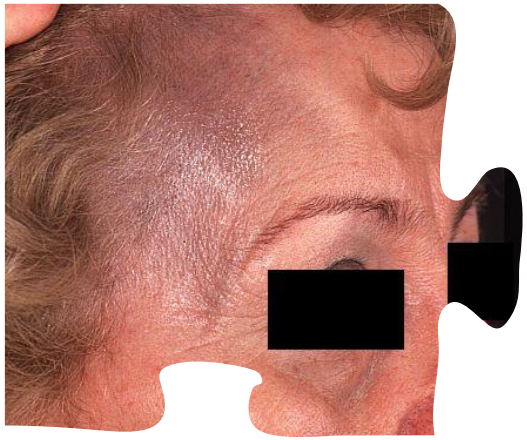
KIT, **NRAS**, BRAF

KIT, **CCND1**, BRAF, NRAS

KIT, NRAS, CDK4

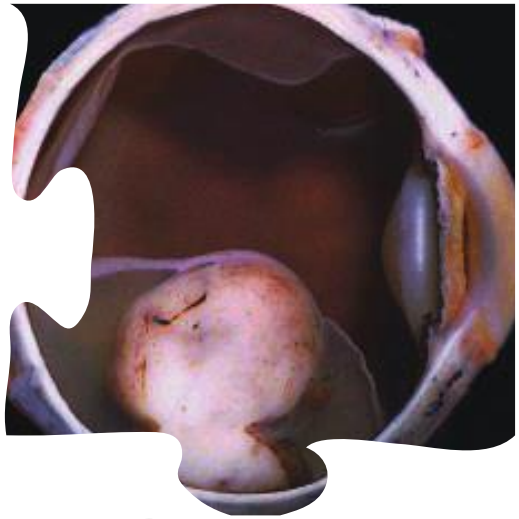
Curtin JA et al, NEJM 2005, JCO 2005

Genotype – Phenotype Correlations in Primary Melanomas



GNAQ

GNA11

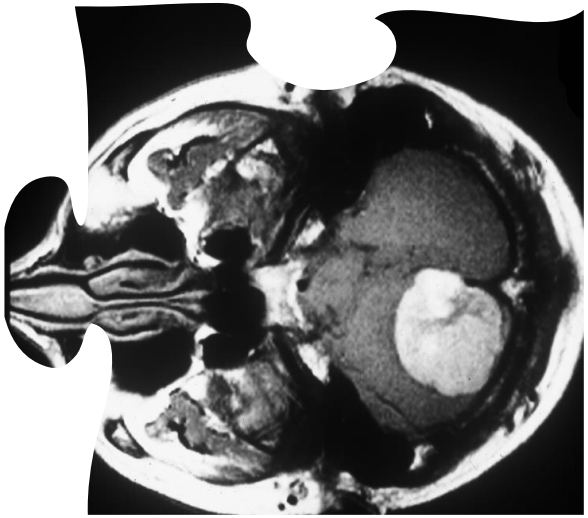


GNAQ

GNA11

GNAQ

GNA11

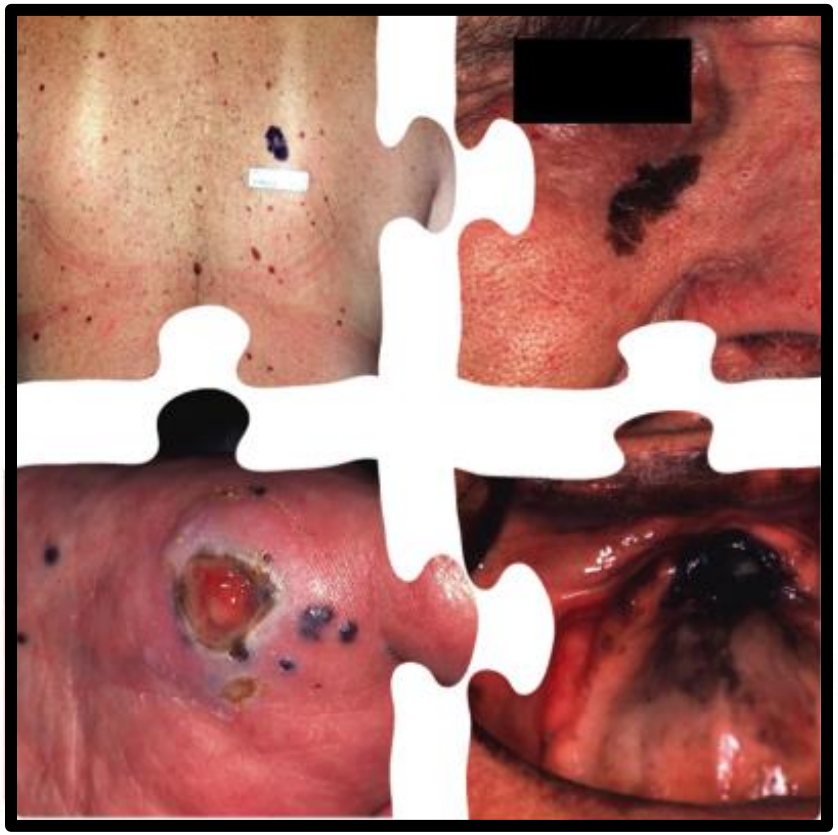


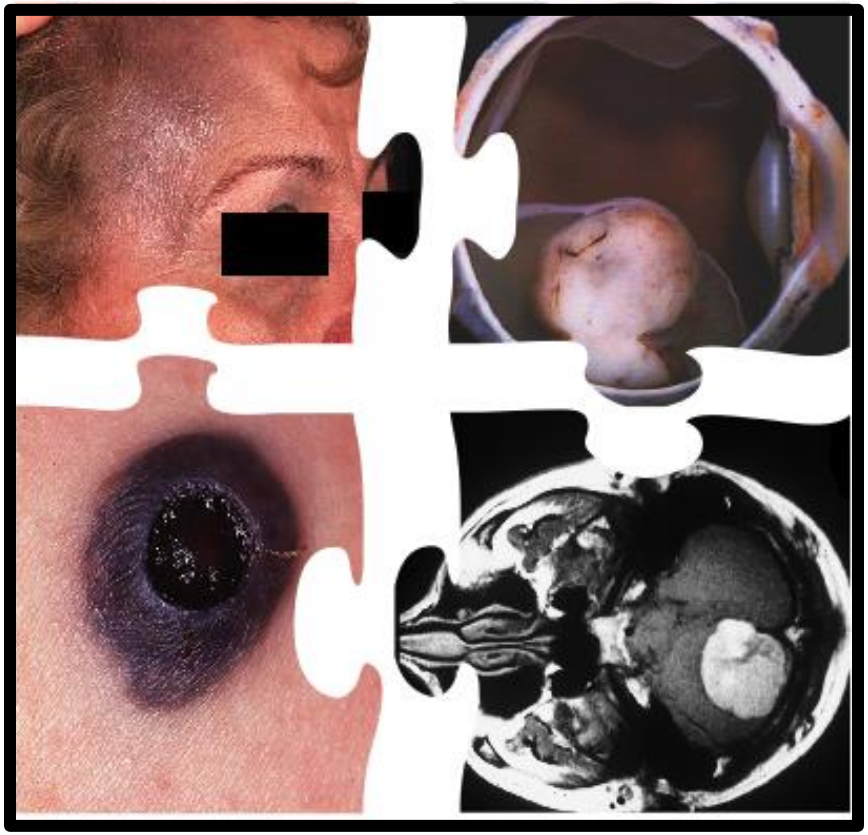
GNAQ

GNA11

Van Raamsdonk et al. Nature 2009, NEJM 2010

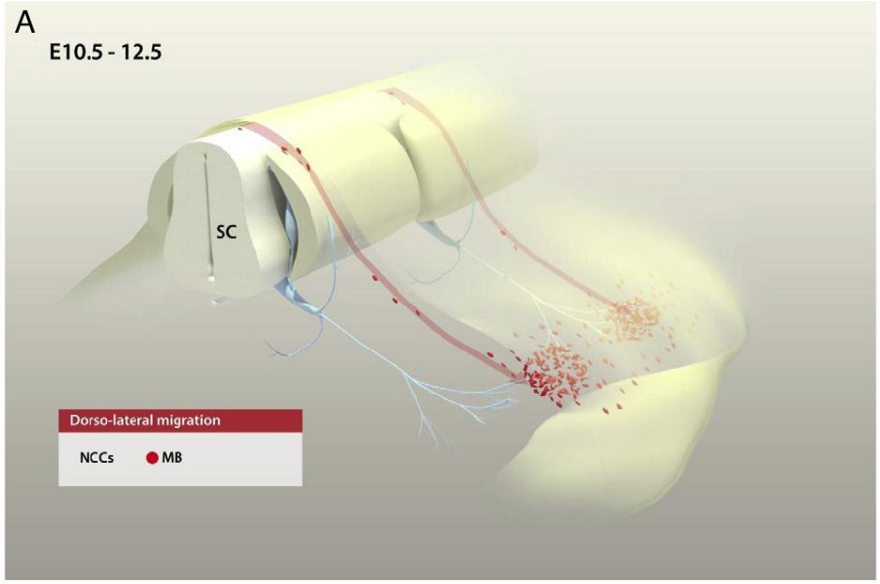
Different subgroups with different cell of origins?



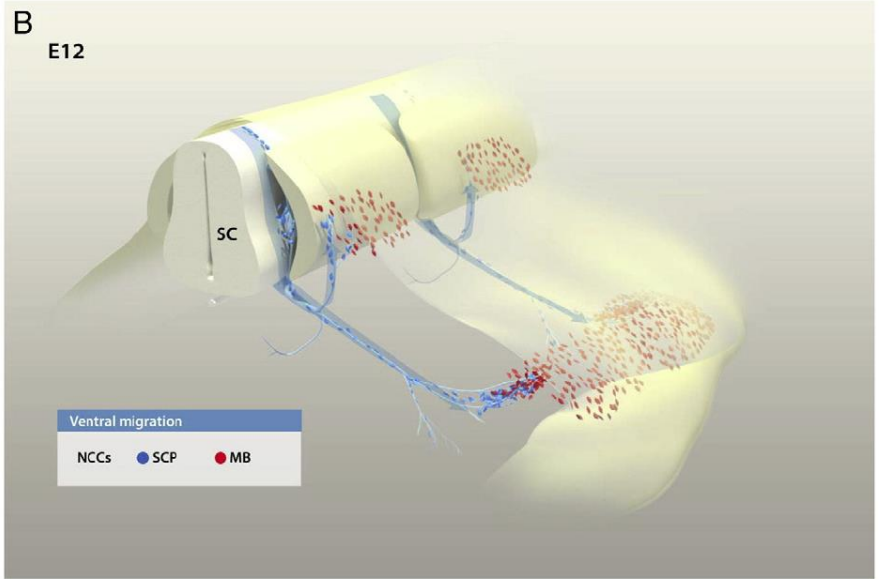


Two pathways for melanocyte development

Dorsolateral



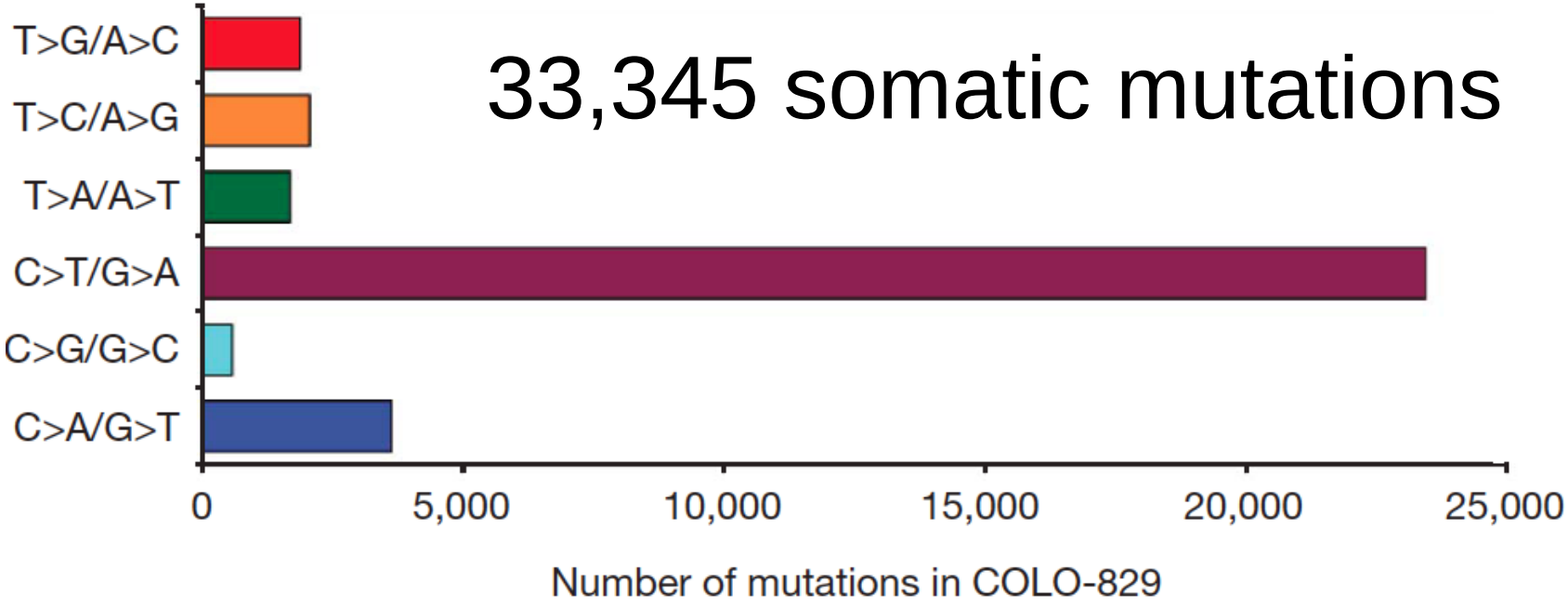
Ventral



Ernfors P
Exp Cell Res. 2010

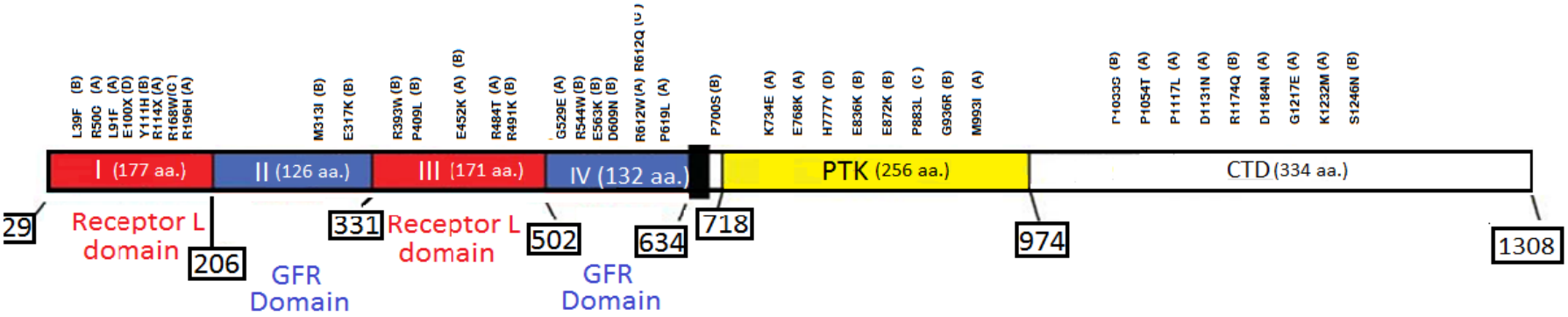
Melanoma gene discovery in the era of massive parallel sequencing

Melanomas on sun-exposed sites have a high mutation burden in their genomes



ED Pleasance *et al. Nature* 000, 1-6 (2009)

High background mutation rate can lead to false postives



e.g. ERBB4

A: Mutations found in this study.
B: Prickett et al.2009
C: Nikolaev et al, 2012
D: Stark et al, 2012

Criteria for separating drivers from passengers

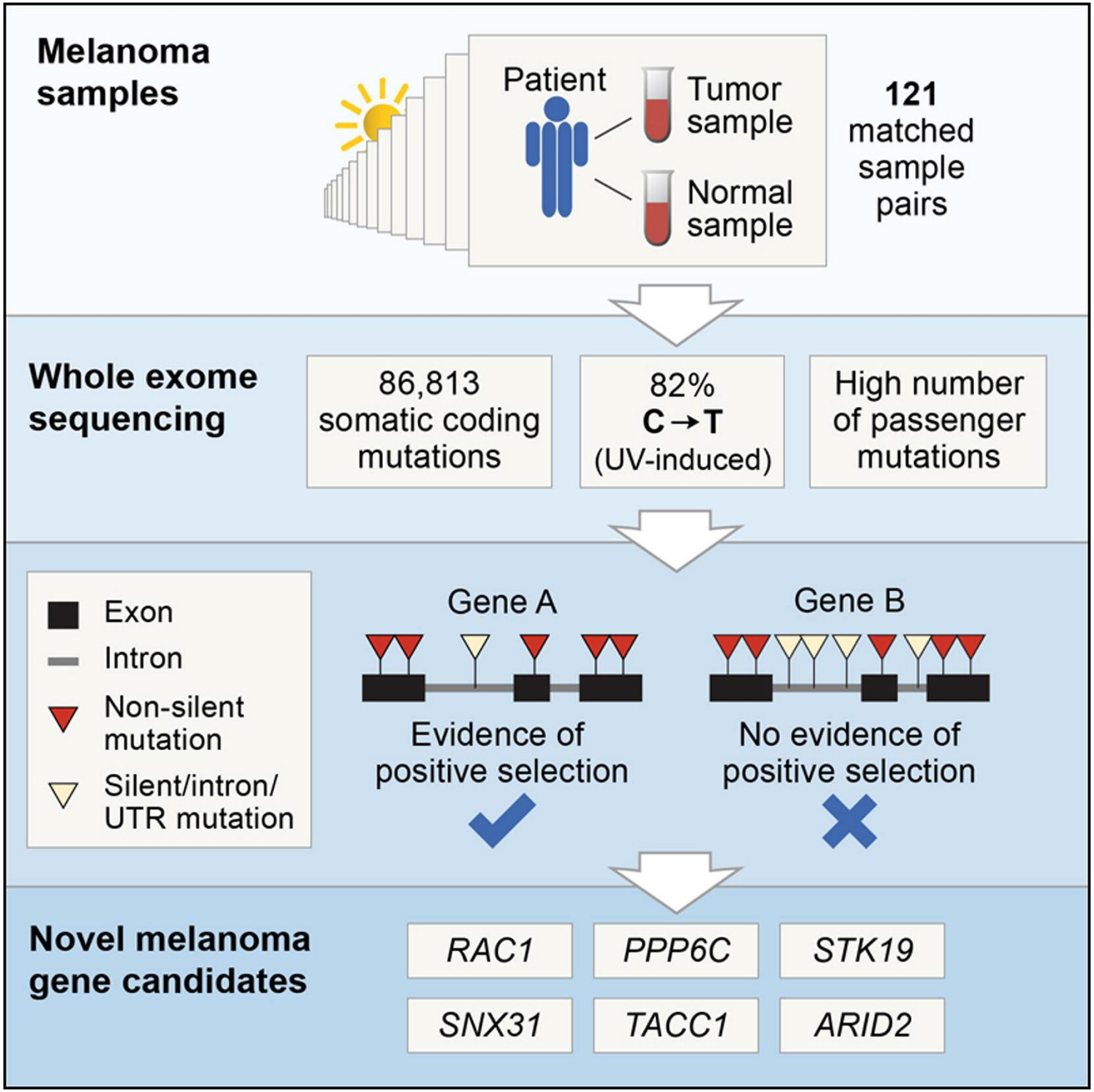
- Background mutation rate
- Gene size
- Level of transcription
- Ratio of non-synonymous to synonymous mutations
- Clustering of mutations at critical functional sites
- Bias in the effect of mutations on the protein

A Landscape of Driver Mutations in Melanoma

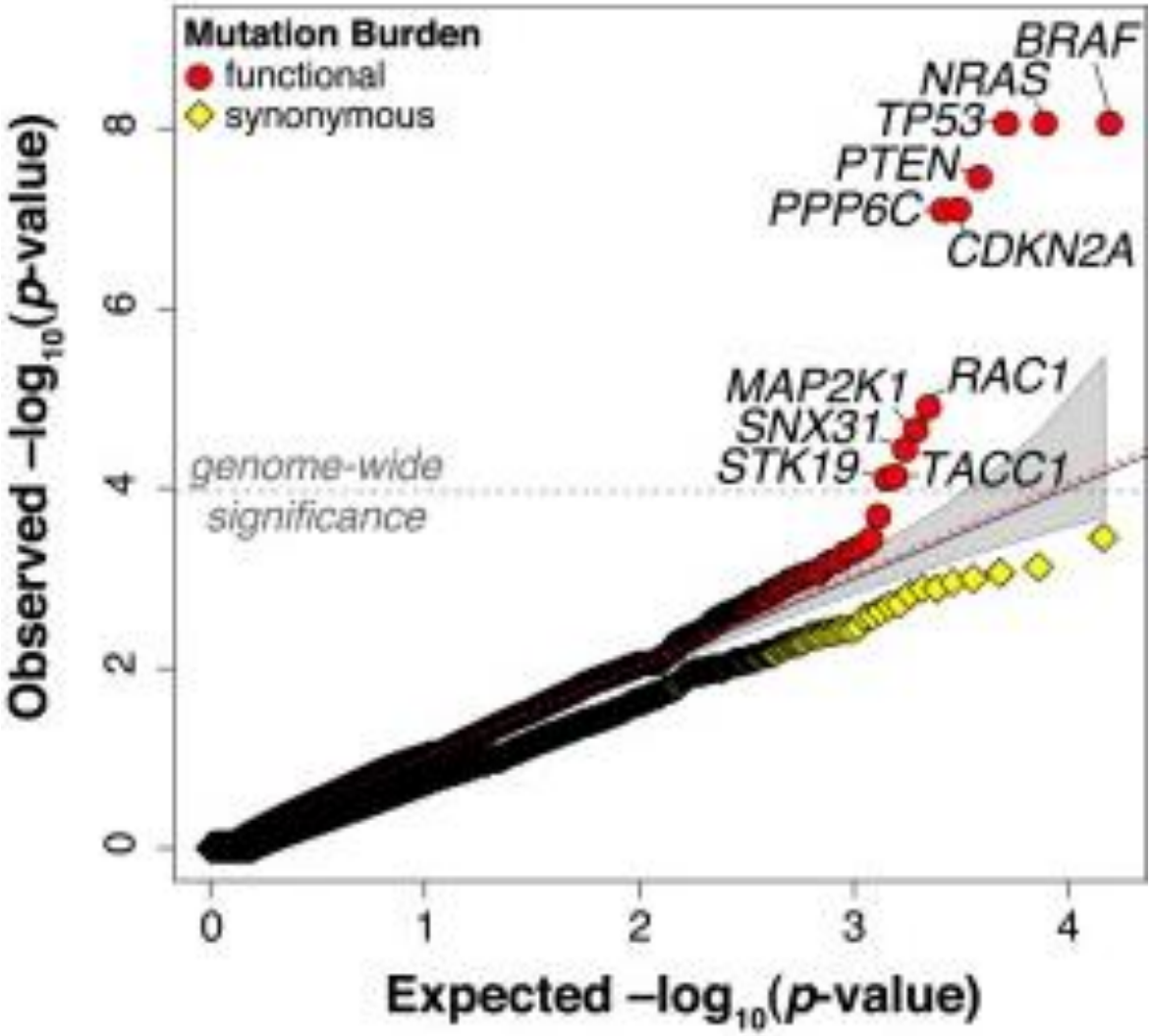
Eran Hodis,[1,2,19] Ian R. Watson,[3,10,19] Gregory V. Kryukov,[1,2,12] Stefan T. Arold,[4] Marcin Imielinski,[1] Jean-Philippe Theurillat,[1] Elizabeth Nickerson,[1] Daniel Auclair,[1] Liren Li,[3,10] Chelsea Place,[10] Daniel DiCara,[1] Alex H. Ramos,[1,2] Michael S. Lawrence,[1] Kristian Cibulskis,[1] Andrey Sivachenko,[1] Douglas Voet,[1] Gordon Saksena,[1] Nicolas Stransky,[1] Robert C. Onofrio,[1] Wendy Winckler,[1] Kristin Ardlie,[1] Nikhil Wagle,[1,2] Jennifer Wargo,[13] Kelly Chong,[14] Donald L. Morton,[15] Katherine Stemke-Hale,[5] Guo Chen,[6] Michael Noble,[1] Matthew Meyerson,[1,2,10,11] John E. Ladbury,[4] Michael A. Davies,[5,6] Jeffrey E. Gershenwald,[7,8] Stephan N. Wagner,[16] Dave S.B. Hoon,[14] Dirk Schadendorf,[17] Eric S. Lander,[1,18] Stacey B. Gabriel,[1] Gad Getz,[1] Levi A. Garraway,[1,2,10,11,20,*] and Lynda Chin[1,2,3,9,10,20,*]

Exome sequencing identifies recurrent somatic *RAC1* mutations in melanoma

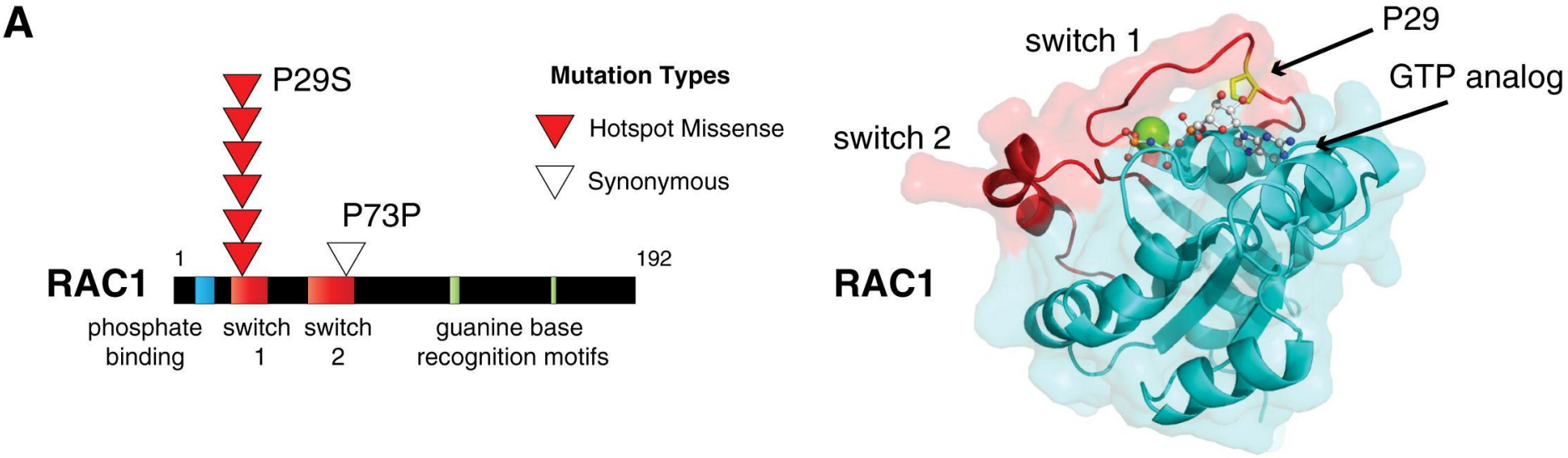
Michael Krauthammer[1], Yong Kong[2,3], Byung Hak Ha[4], Perry Evans[1], Antonella Bacchiocchi[5], James P McCusker[1], Elaine Cheng[5], Matthew J Davis[4], Gerald Goh[6,7], Murim Choi[6,7], Stephan Ariyan[8], Deepak Narayan[8], Ken Dutton-Regester[9,10], Ana Capatana[1], Edna C Holman[5], Marcus Bosenberg[5], Mario Sznol[11], Harriet M Kluger[11], Douglas E Brash[5,6,12], David F Stern[1], Miguel A Materin[13], Roger S Lo[14], Shrikant Mane[6,15,16], Shuangge Ma[17], Kenneth K Kidd[6], Nicholas K Hayward[10], Richard P Lifton[6,7], Joseph Schlessinger[4], Titus J Boggon[4] & Ruth Halaban[5]

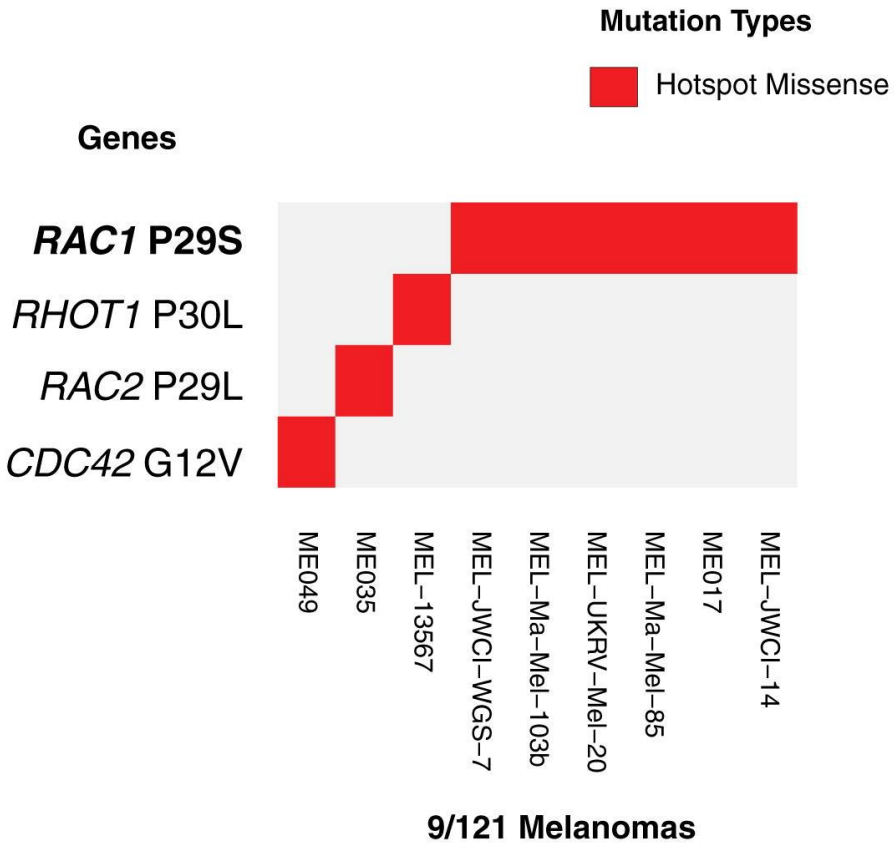

Melanoma samples
Patient
Tumor sample
Normal sample
121 matched sample pairs
Whole exome sequencing
86,813 somatic coding mutations
82% C→T (UV-induced)
High number of passenger mutations
Exon
Intron
Non-silent mutation
Silent/intron/ UTR mutation
Gene A
Evidence of positive selection
Gene B
No evidence of positive selection
Novel melanoma gene candidates
RAC1
PPP6C
STK19
SNX31
TACC1
ARID2

Significant mutations

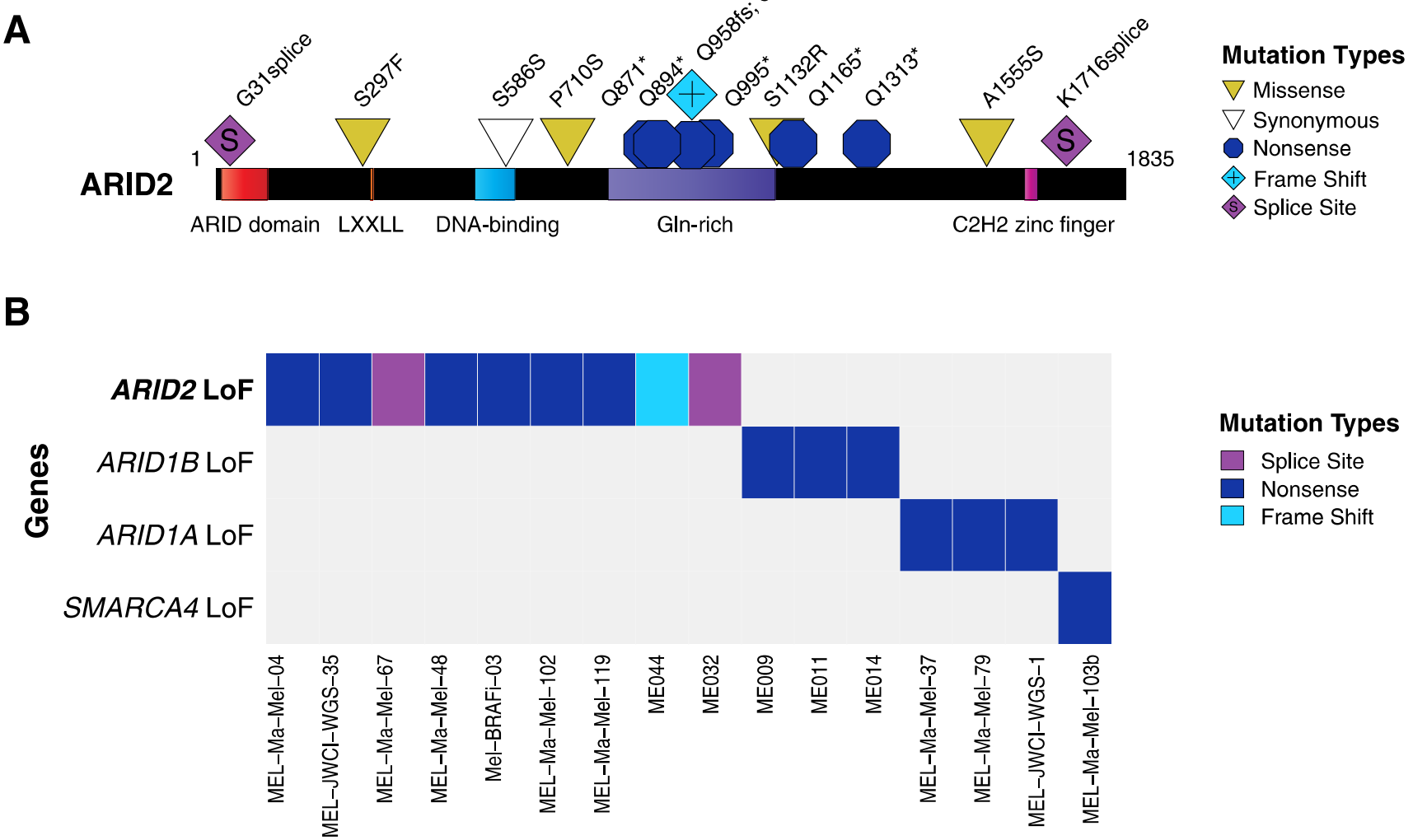


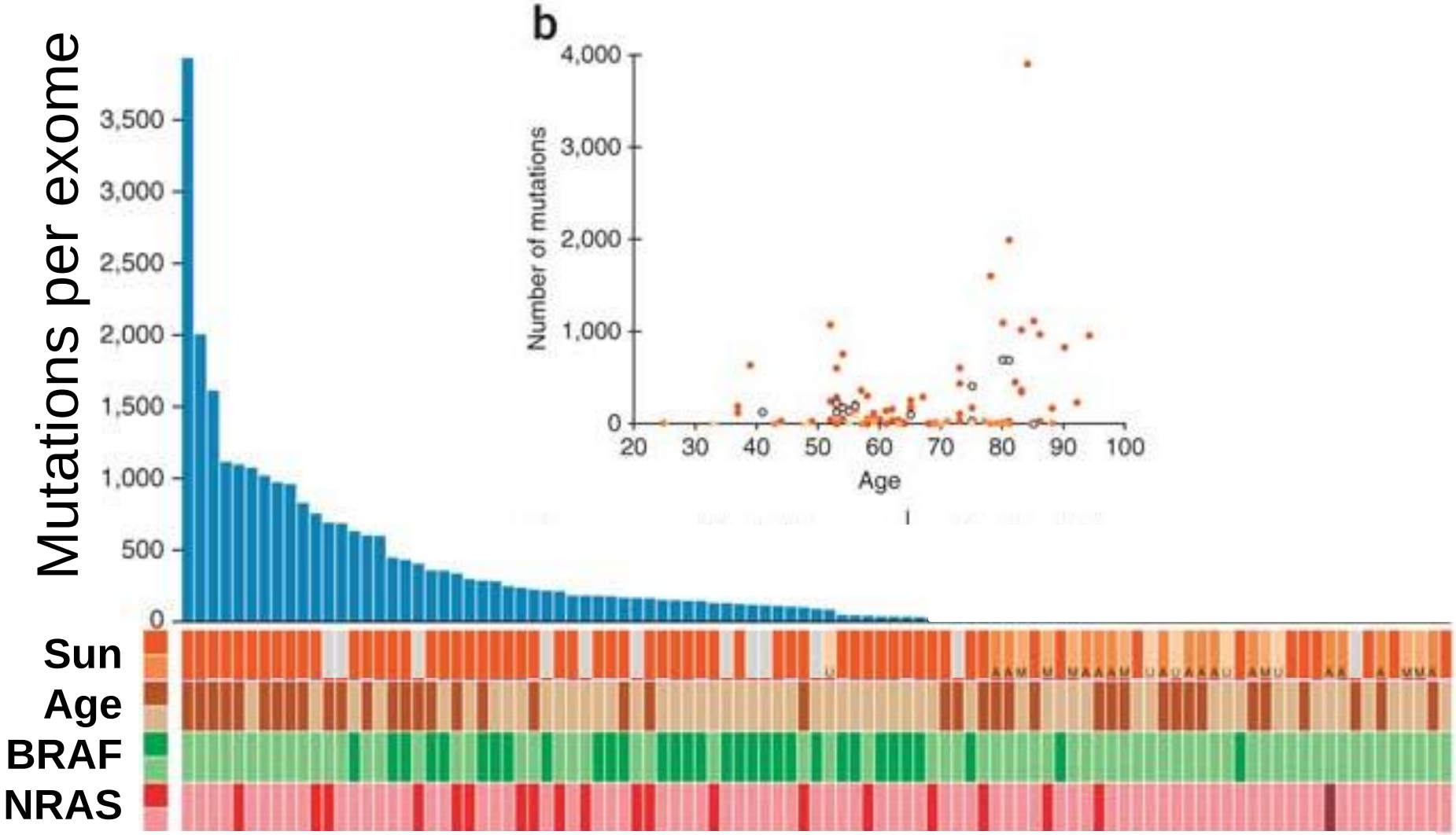
Hodis et al. Cell 2012


A
P29S
Mutation Types
Hotspot Missense
Synonymous
P73P
1
192
RAC1
phosphate binding
switch 1
switch 2
guanine base recognition motifs
switch 1
P29
GTP analog
switch 2
RAC1


B
Mutation Types
Hotspot Missense
Genes
RAC1 P29S
RHOT1 P30L
RAC2 P29L
CDC42 G12V
ME049
ME035
MEL-13567
MEL-JWCI-WGS-7
MEL-Ma-Mel-103b
MEL-UKRV-Mel-20
MEL-Ma-Mel-85
ME017
MEL-JWCI-14
9/121 Melanomas

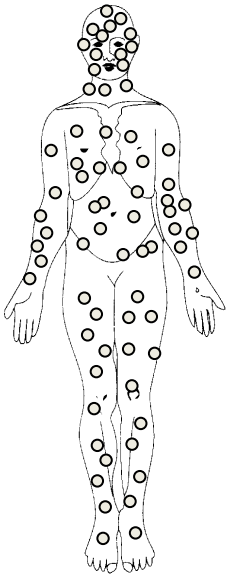
Typical mutation pattern of a tumor suppressor gene





Krauthammer et al. Nat Genet. 2012

The Susceptibility Hypothesis



BRAF dependent

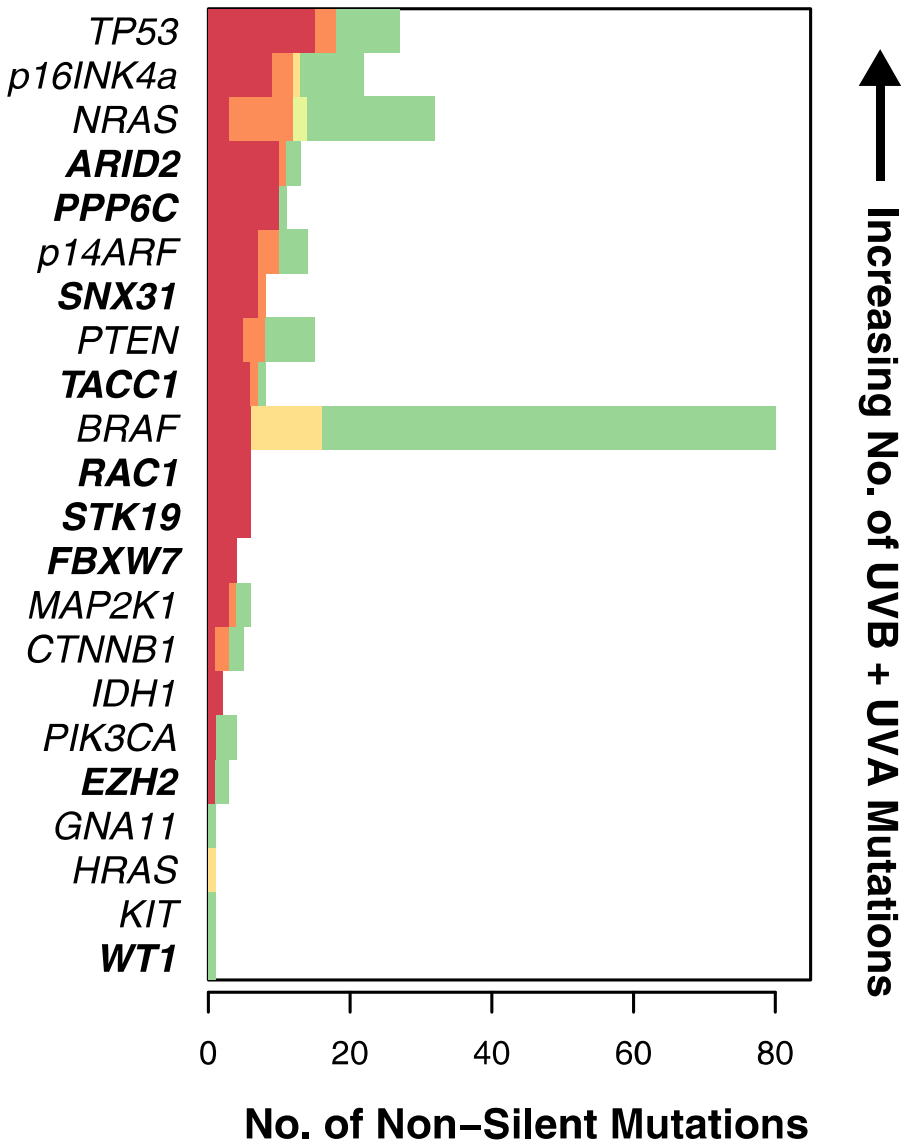
Younger

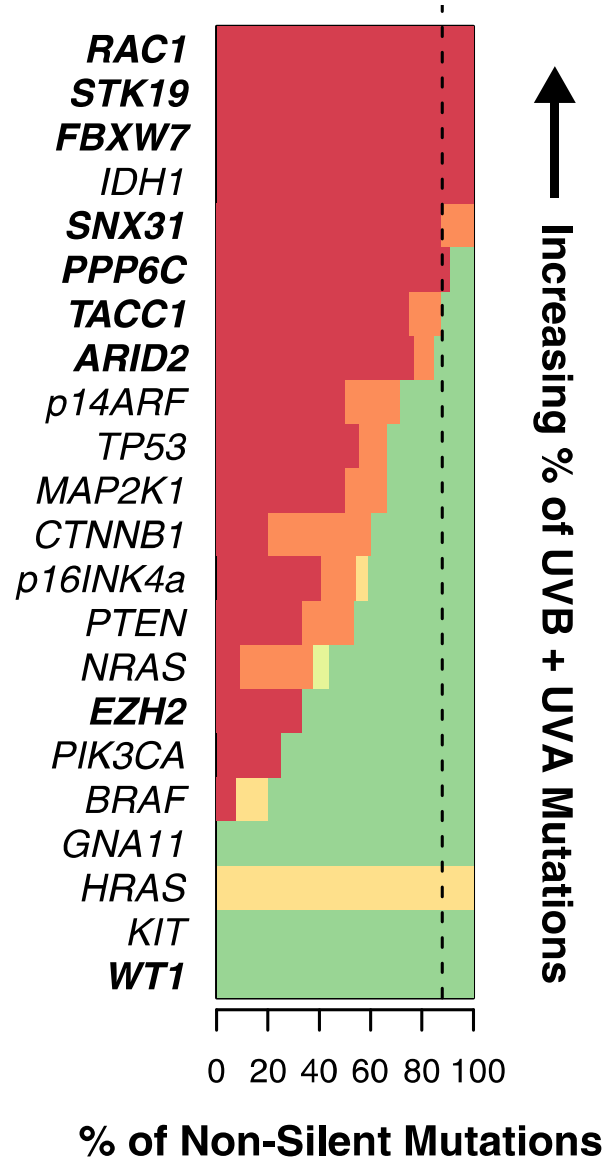
BRAF independent

Older

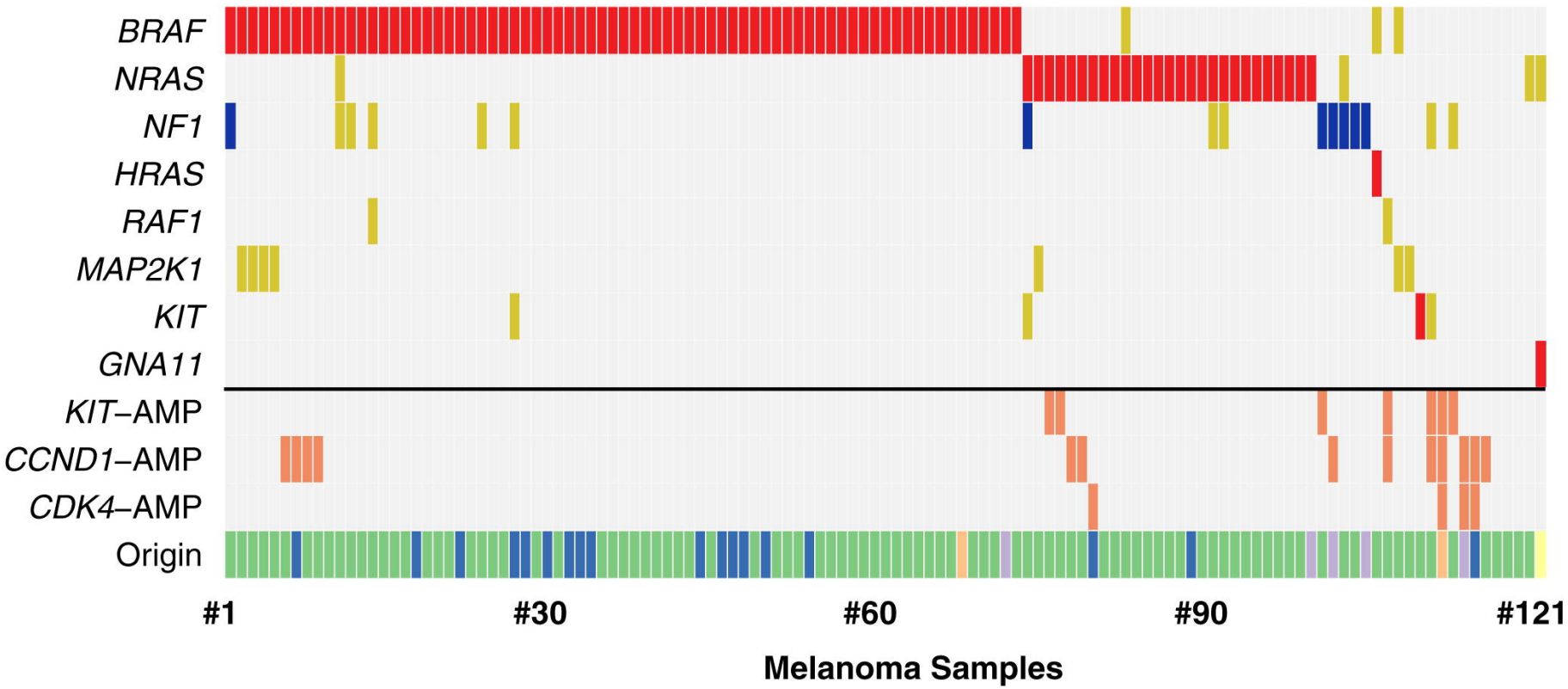
Curtin JA et al, NEJM 2005

UV Signatures





An expanded list of melanoma driver genes



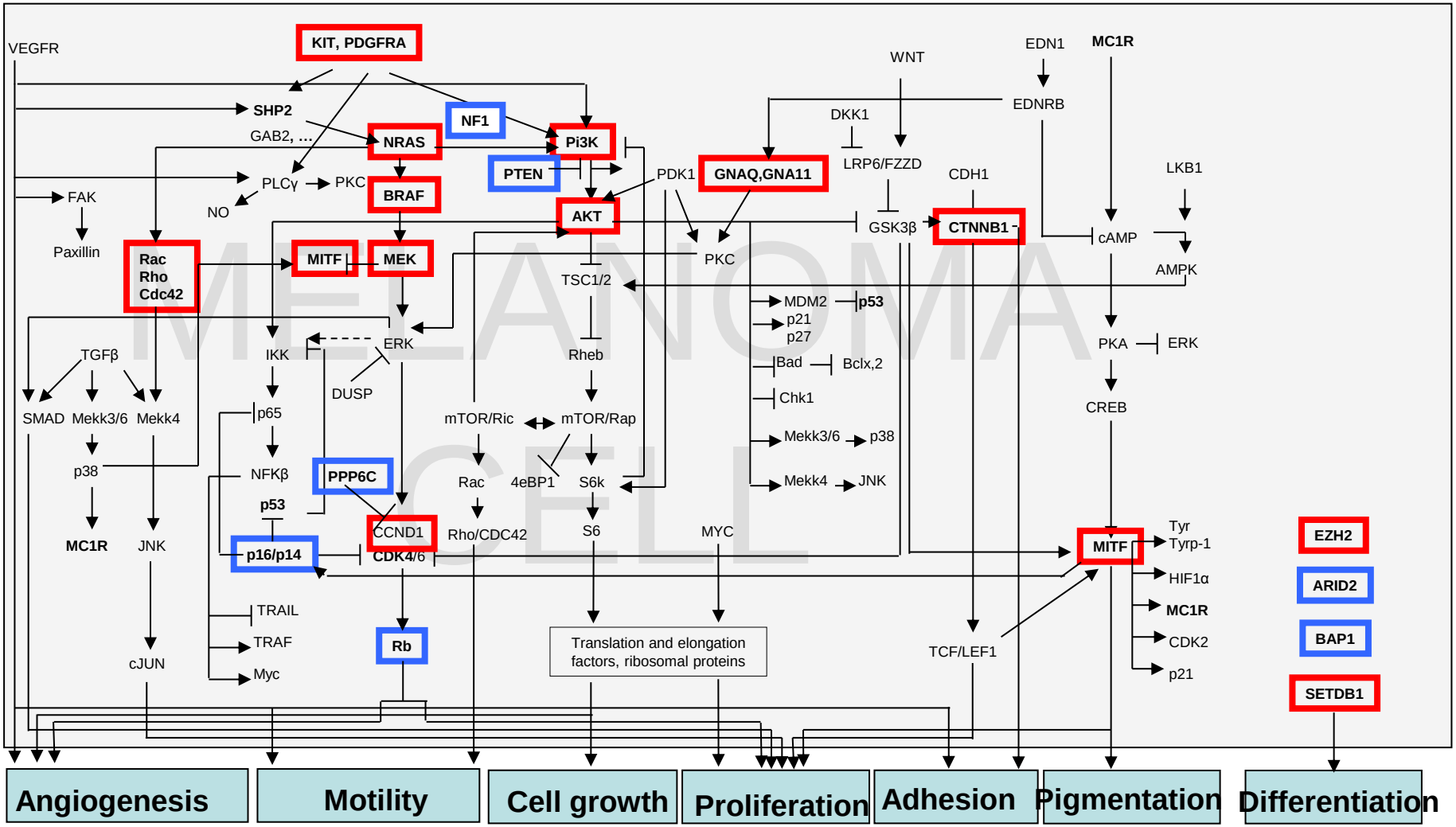
Novel melanoma driver genes

Strong genetic support

- RAC1
- ARID2
- PPP6C
- STK19
- RAF1
- NF1
- MAP2K1
- BAP1

Waning genetic support

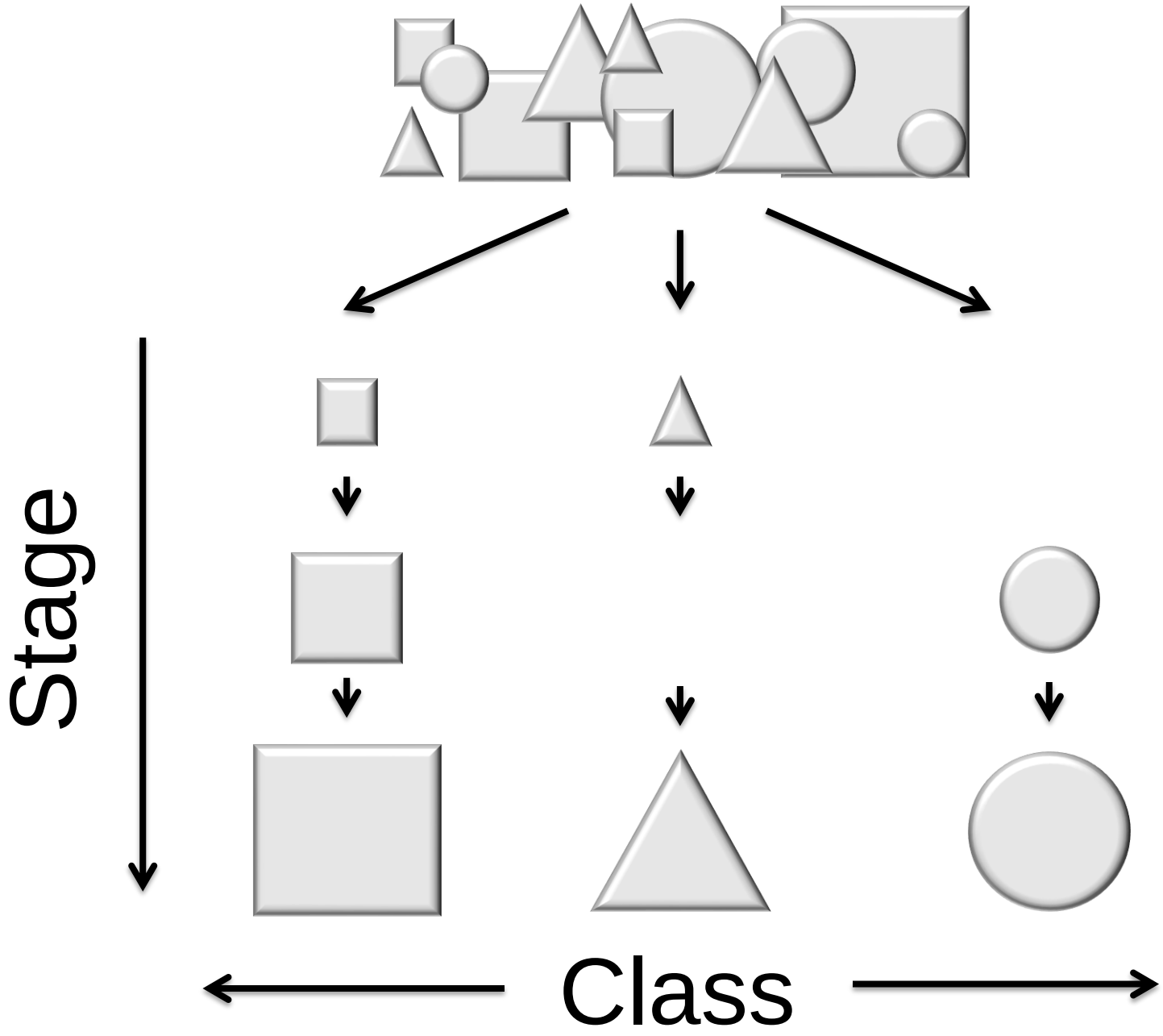
- GRIN2A
- TRRAP
- ERBB4
- PREX2
- MMP8
- GRM3
- MAP3K5
- MAP3K9


MELANOMA CELL
VEGFR
KIT, PDGFRA
SHP2
GAB2, ...
NF1
NRAS
Pi3K
PTEN
PLCγ
PKC
NO
BRAF
FAK
Paxillin
Rac
Rho
Cdc42
MITF
MEK
AKT
PDK1
GNAQ,GNA11
WNT
DKK1
LRP6/FZZD
GSK3β
CTNNB1
EDN1
MC1R
EDNRB
CDH1
LKB1
cAMP
AMPK
PKC
TSC1/2
MDM2
p53
p21
p27
Bad
Bclx,2
Chk1
Mekk3/6
p38
Mekk4
JNK
PKA
ERK
CREB
TGFβ
SMAD
Mekk3/6
Mekk4
p38
MC1R
JNK
cJUN
IKK
ERK
DUSP
p65
NFKβ
p53
PPP6C
p16/p14
CCND1
CDK4/6
Rb
TRAIL
TRAF
Myc
Rheb
mTOR/Ric
mTOR/Rap
Rac
4eBP1
S6k
Rho/CDC42
S6
MYC
Translation and elongation factors, ribosomal proteins
TCF/LEF1
MITF
Tyr
Tyrp-1
HIF1α
MC1R
CDK2
p21
EZH2
ARID2
BAP1
SETDB1
Angiogenesis
Motility
Cell growth
Proliferation
Adhesion
Pigmentation
Differentiation

Validating genotype-phenotype correlations experimentally

GNAQ^{Q209L}

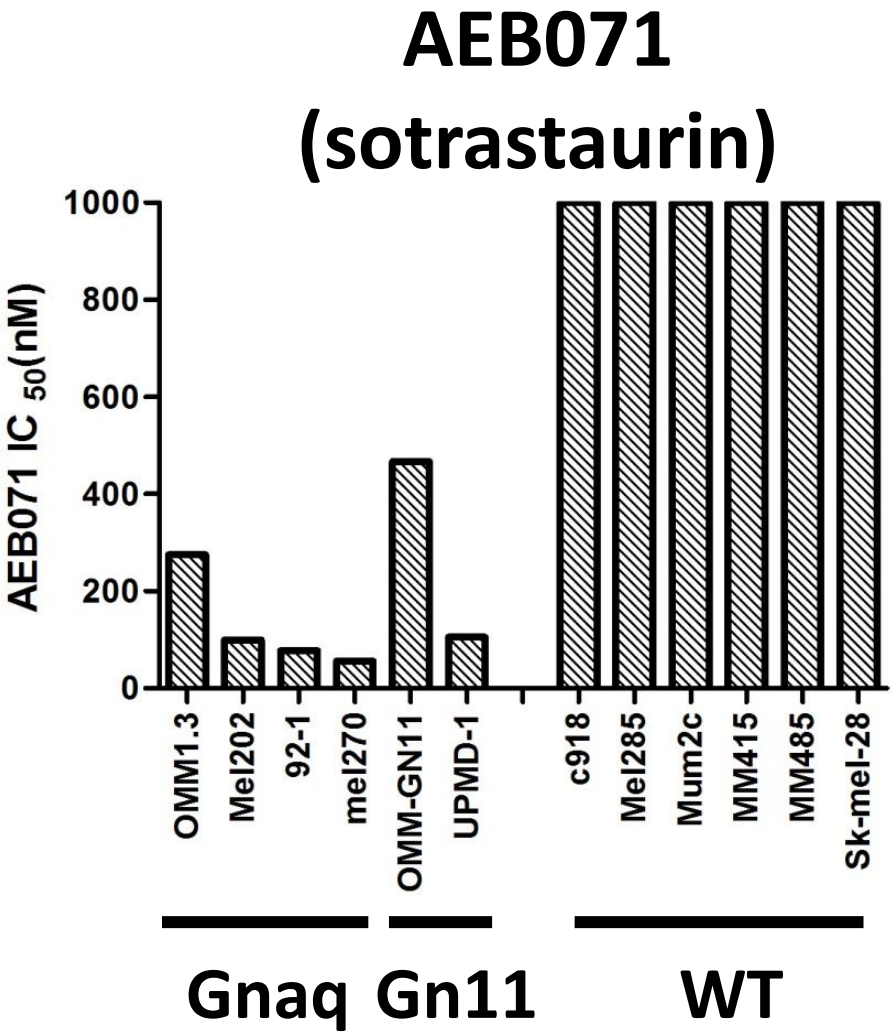
NRAS^{G12V}

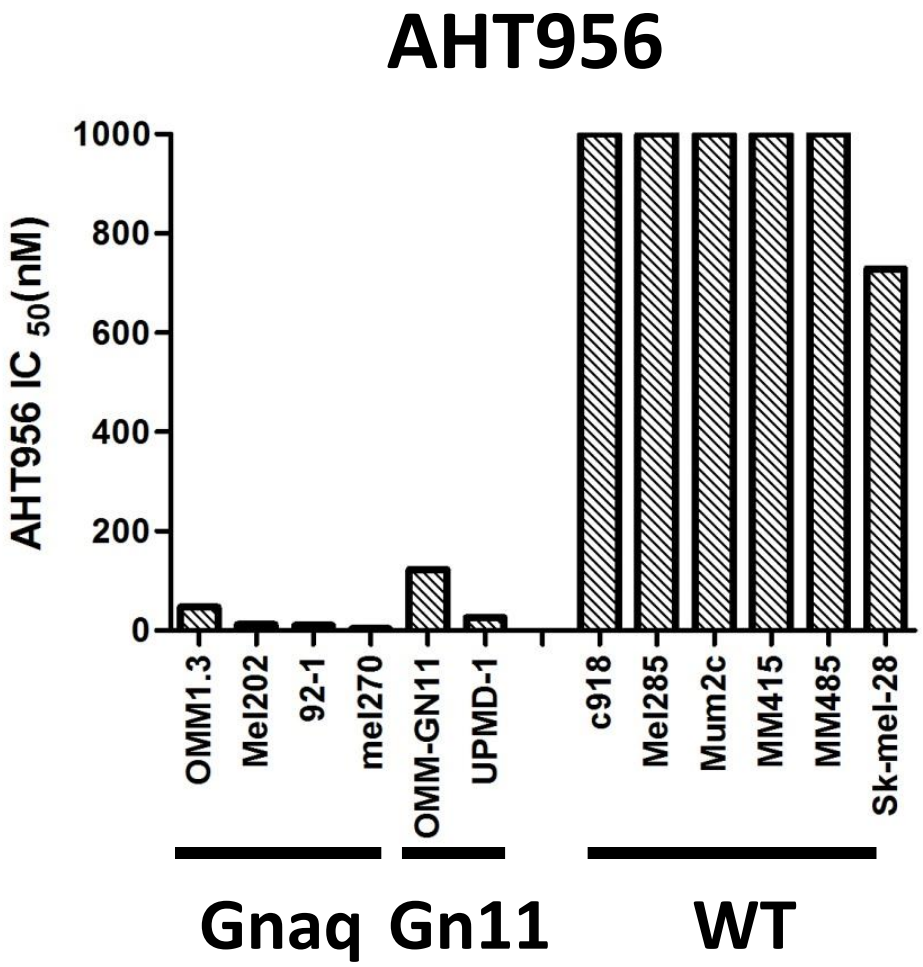
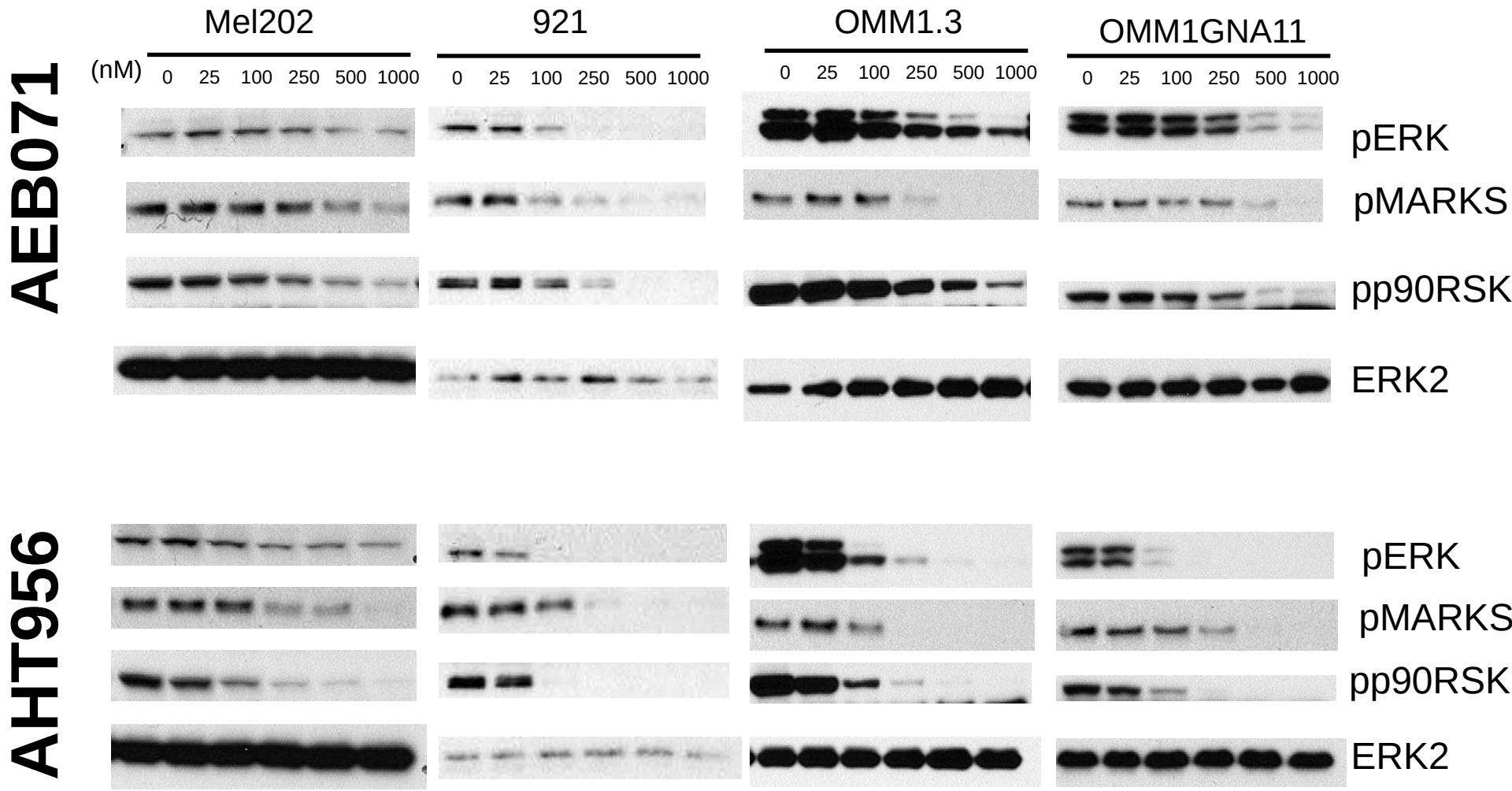

Stage
Class

Conclusions

- Melanomas are a genetically and phenotypically diverse group of biologically distinct entities
- Integrating molecular and phenotypic features can lead to an improved, more clinically relevant taxonomy

Selective sensitivity of human melanoma cell lines with Gnaq/11 mutation to PKC inhibitor





PKC inhibitors suppress $G_{q/11}$ signaling in a dosedependent manner in human uveal melanoma cell lines with GNAQ or GNA11 mutations