



**Comprehensive characterization
of the adrenal cortical cancer
genome**

Adrenal Research Working Group

Siyuan Zheng, Ph.D.

szheng2@mdanderson.org

MD Anderson Cancer Center

Adrenal Cortical Carcinoma is a rare cancer

- Annual incidence is between 0.5-2 per million
- 5 year survival for patients with metastasis is less than 20%
- No standard staging system
- Endocrine tumor, with more than half patients have hormone excess
- Mitotane is the only FDA approved drug.
- 2 Targeted therapy so far is disappointing

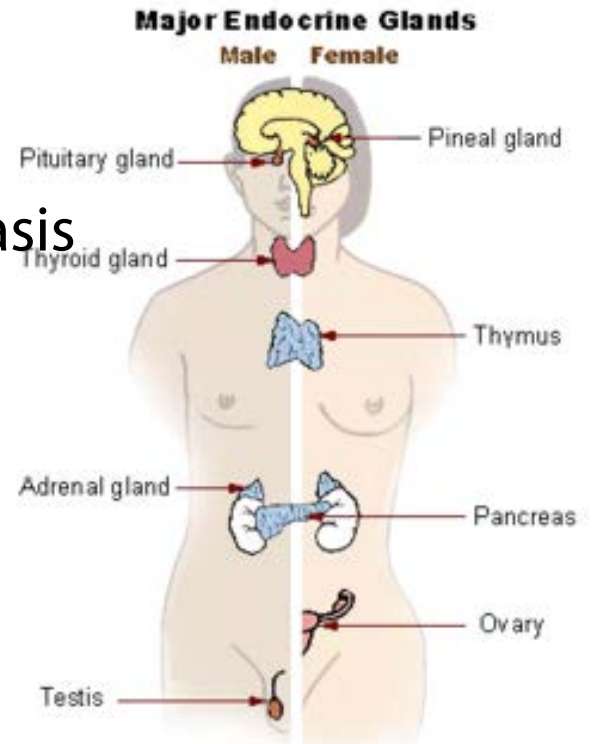


Image from wikipedia

TCGA: Samples collected for genomic characterization



Platform	Method	Center	Number of Samples
Mutations	Exome sequencing	Baylor (Houston, TX)	91
Transcriptome	RNA sequencing	UNC (Chapel Hill, VA)	79
microRNA	microRNA sequencing	UNC (Vancouver, BC)	80
DNA copy number	Affymetrix SNP6 arrays	Broad Institute (Cambridge, MA)	90
Methylation	Illumina 450k BeadArrays	USC (Los Angeles, CA)	80
Proteome	Reverse phase protein arrays	MDACC (Houston, TX)	68*
Clinical			92**

* Samples to be shipped

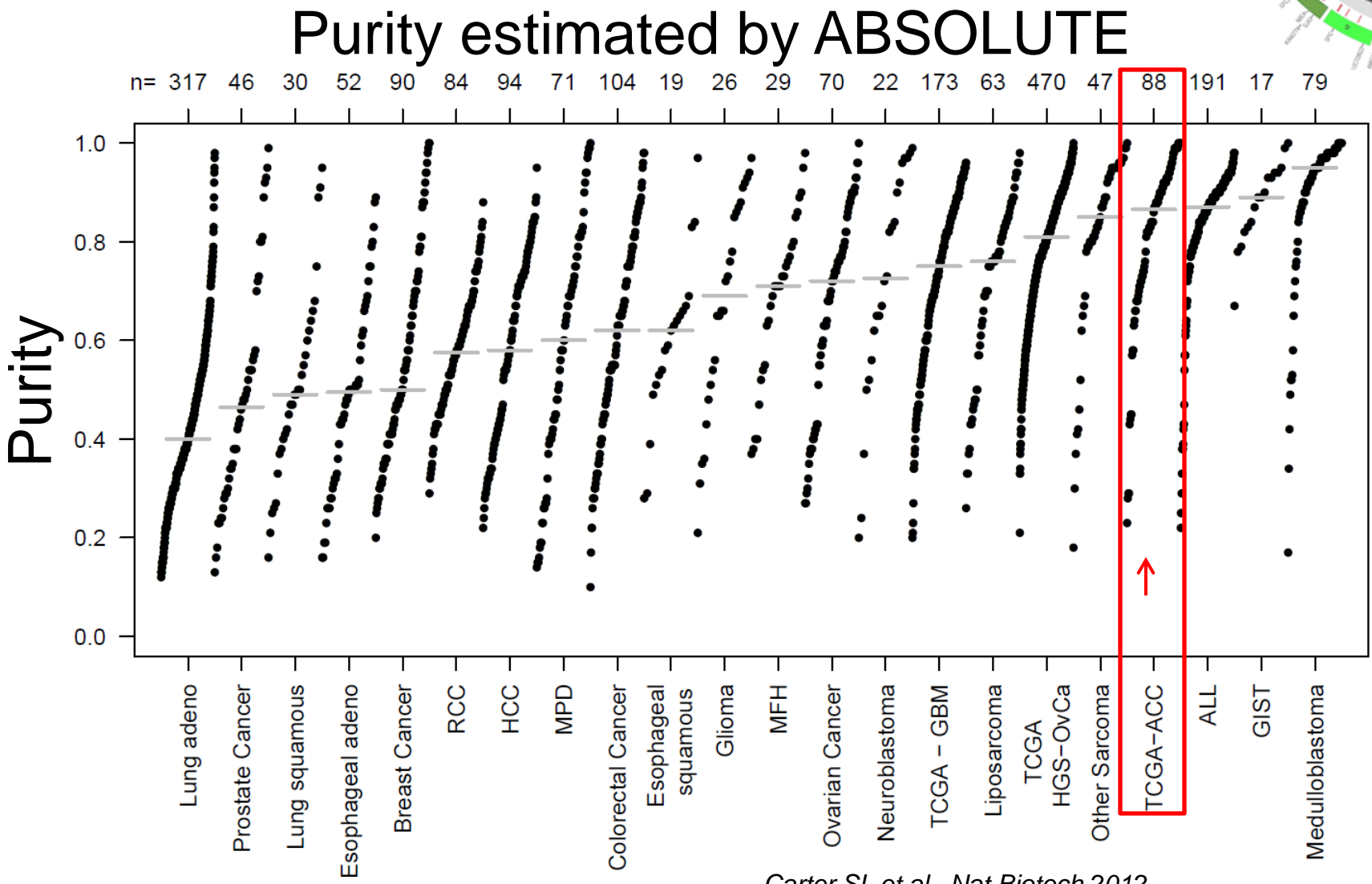
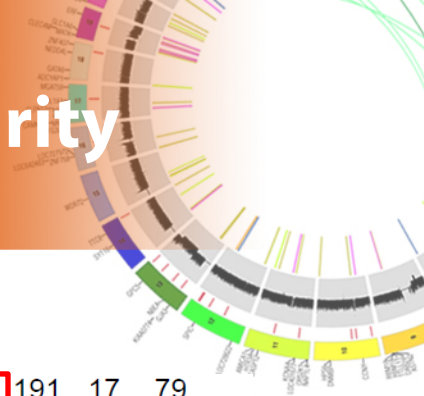
** Clinical data mostly pending

TCGA ACC project



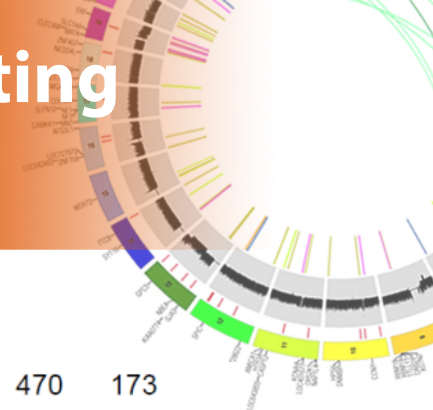
- Analysis working group started Oct 2013
- Data generation completed Dec 2013
- Current status: First pass analysis complete, more advanced analysis underway
 - Expression clustering
 - Methylation clustering
 - Significant gene mutations
 - DNA Copy nr alterations
 - Transcript fusions
 - Integrated data analysis

ACC specimen show relatively high tumor purity

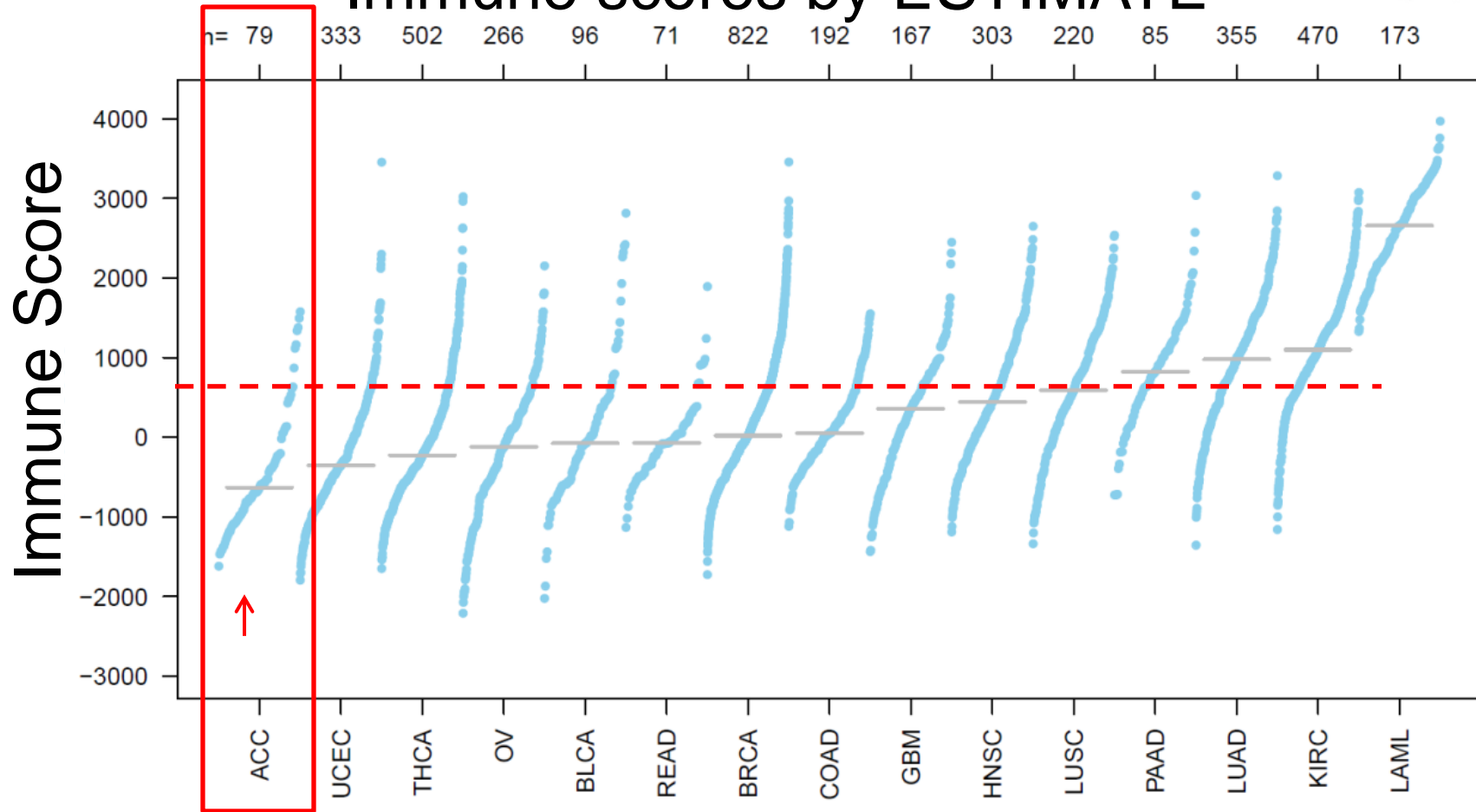


Carter SL et al., Nat Biotech 2012

A subset of ACC may be related to infiltrating leukocytes

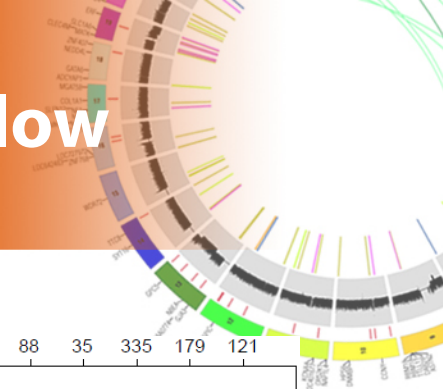


Immune scores by ESTIMATE

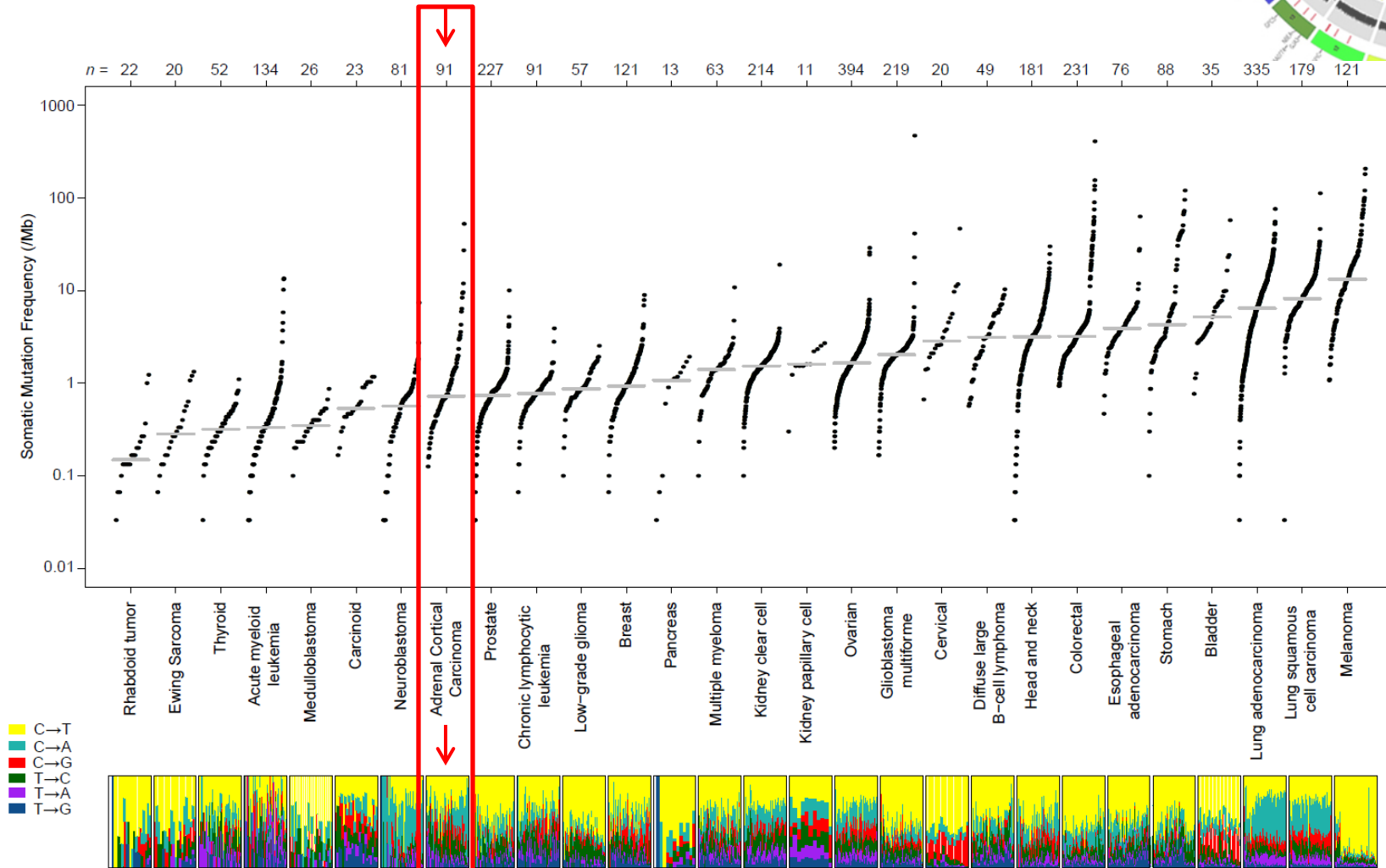


Yoshihara K., Nat Communications 2013 PMID: 24113773

The mutation frequency of ACC is relatively low



Mutation Frequency



Analysis of 91 ACC exomes identifies five significantly mutated genes



Gene	reptime	n_nonsilent	n_silent	p	q	COSMIC
TP53	213	16	1	0	0	1
CTNNB1	448	13	0	3.03E-08	2.86E-04	1
MEN1	230	6	0	7.25E-07	4.56E-03	1
PRKAR1A	313	5	0	1.91E-06	9.02E-03	1
RPL22	240	3	0	2.02E-05	7.63E-02	1
ZNRF3	326	4	0	5.37E-03	1	0
MDM2	228	2	0	2.35E-02	1	1
RB1	450	2	0	2.60E-02	1	1

PRKAR1A is a binding partner of PRKACA and this complex induces cortisol production and proliferation



The NEW ENGLAND JOURNAL of MEDICINE
Constitutive Activation of PKA Catalytic Subunit in Adrenal Cushing's Syndrome

Felix Beuschlein, M.D., Martin Fassnacht, M.D., Guillaume Assié, M.D., Ph.D., Davide Calebiro, M.D., Ph.D., Constantine A. Stratakis, M.D., D.Sc., Andrea Osswald, M.D., Cristina L. Ronchi, M.D., Ph.D., Thomas Wieland, M.Sc., Silviu Sbiera, Ph.D., Fabio R. Faucz, Ph.D., Katrin Schaak, Ph.D., Anett Schmittfull, M.S., Thomas Schwarzmayr, M.Sc., Olivia Barreau, M.D., Ph.D., Delphine Vezzosi, M.D., Ph.D., Marthe Rizk-Rabin, Ph.D., Ulrike Zabel, Ph.D., Eva Szarek, Ph.D., Paraskevi Salpea, Ph.D., Antonella Forlino, Ph.D., Annalisa Vetro, Ph.D., Orsetta Zuffardi, Ph.D., Caroline Kisker, Ph.D., Susanne Diener, M.Sc., Thomas Meitinger, M.D., Martin J. Lohse, M.D., Martin Reincke, M.D., Jérôme Bertherat, M.D., Ph.D., Tim M. Strom, M.D., and Bruno Allolio, M.D.

N ENGL J MED 370;11 NEJM.ORG MARCH 13, 2014

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Recurrent activating mutation in *PRKACA* in cortisol-producing adrenal tumors

Gerald Goh^{1,2}, Ute I Scholl¹⁻³, James M Healy⁴, Murim Choi^{1,2,5}, Manju L Prasad⁶, Carol Nelson-Willia⁷, John W Kuntsman⁴, Reju Korah⁴, Anna-Carina Suttorp⁷, Dimo Dietrich⁸, Matthias Haase⁹, Holger S Willenberg⁹, Peter Stålberg¹⁰, Per Hellman¹⁰, Göran Åkerström¹⁰, Peyman Björklund¹⁰, Tobias Carling^{4,11} & Richard P Lifton^{1,2,5}

nature
genetics

Activating Hotspot L205R Mutation in *PRKACA* and Adrenal Cushing's Syndrome

Yanan Cao,^{1†} Minghui He,^{2†} Zhibo Gao,^{2†} Ying Peng,¹ Yanli Li,¹ Lin Li,² Weiwei Zhou,¹ Xiangchun Li,² Xu Zhong,¹ Yiming Lei,² Tingwei Su,¹ Hang Wang,² Yiran Jiang,¹ Lin Yang,² Wei Wei,¹ Xu Yang,² Xiuli Jiang,¹ Li Liu,² Juan He,¹ Junna Ye,¹ Qing Wei,⁴ Yingrui Li,² Weiqing Wang,^{1*} Jun Wang,^{2,5,6,7,8*} Guang Ning^{1,3*}

3 April 2014 / Page 1 / 10.1126/science.1249480

Science

AAAS

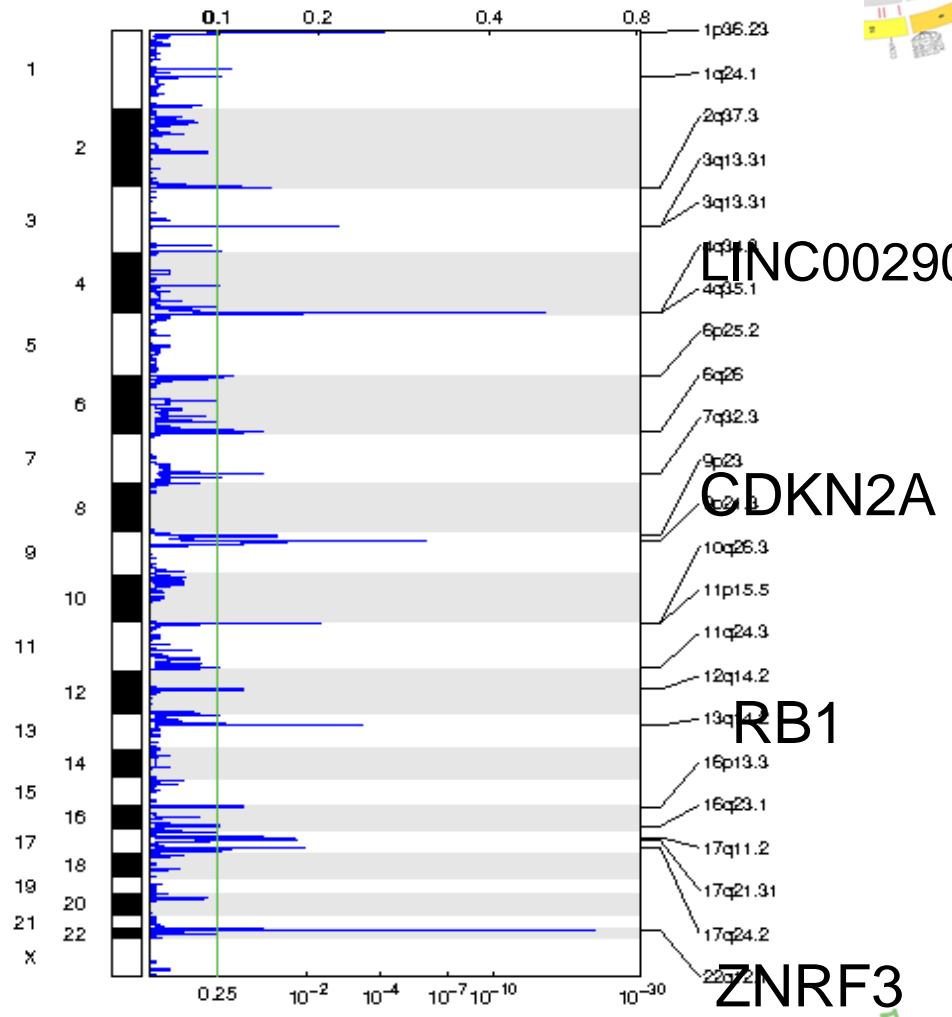
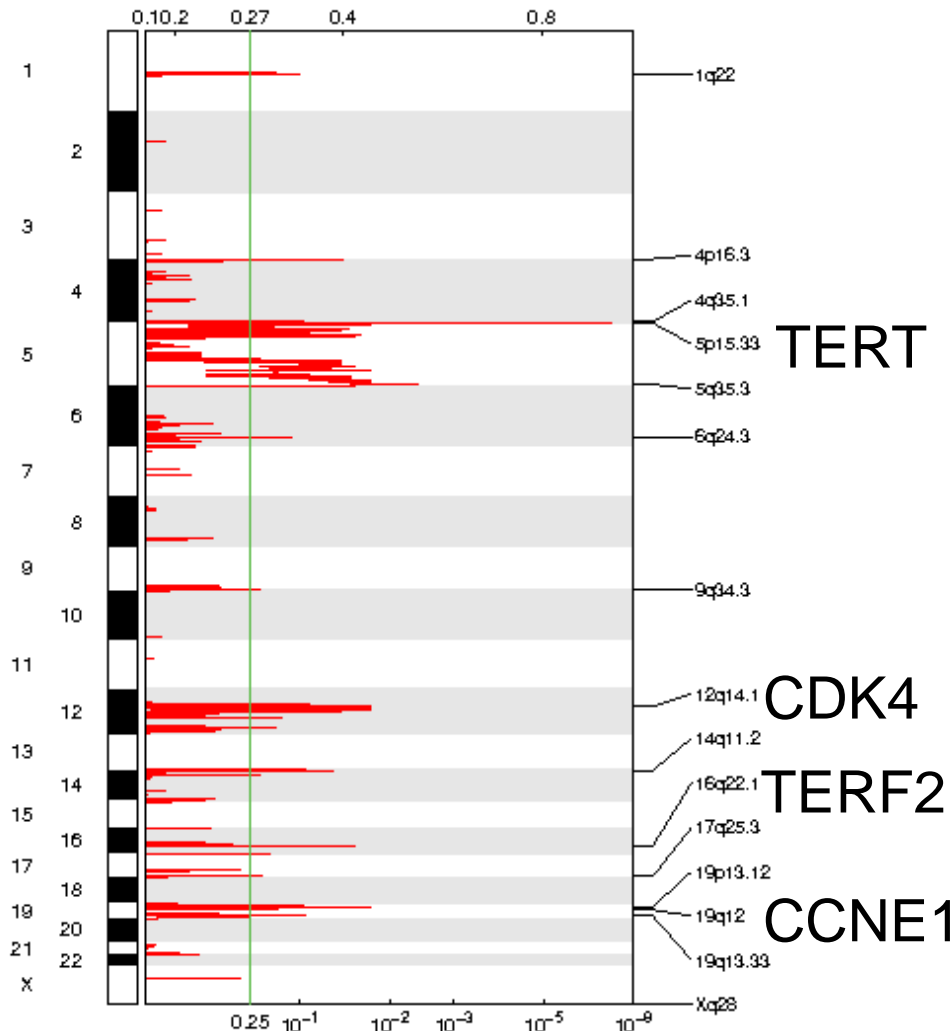
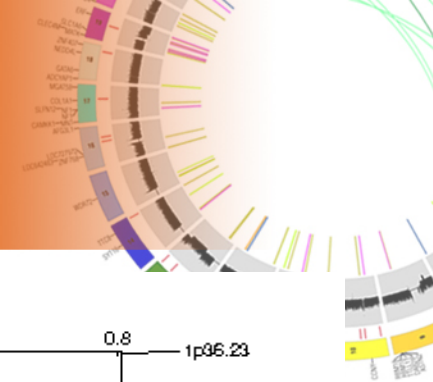
Detection of a Recurrent *DNAJB1-PRKACA* Chimeric Transcript in Fibrolamellar Hepatocellular Carcinoma

Joshua N. Honeyman,^{1,2*} Elana P. Simon,^{1,3*} Nicolas Robine,^{4*} Rachel Chiaroni-Clarke,¹ David G. Darcy,^{1,2} Irene Isabel P. Lim,^{1,2} Caroline E. Gleason,¹ Jennifer M. Murphy,^{1,2} Brad R. Rosenberg,⁵ Lydia Teegan,¹ Constantin N. Takacs,¹ Sergio Botero,¹ Rachel Belote,¹ Soren Germer,⁴ Anne-Katrin Emde,⁴ Vladimir Vacic,⁴ Umesh Bhanot,⁶ Michael P. LaQuaglia,² Sanford M. Simon^{1†}

Science 343, 1010 (2014);

The Cancer Genome Atlas 

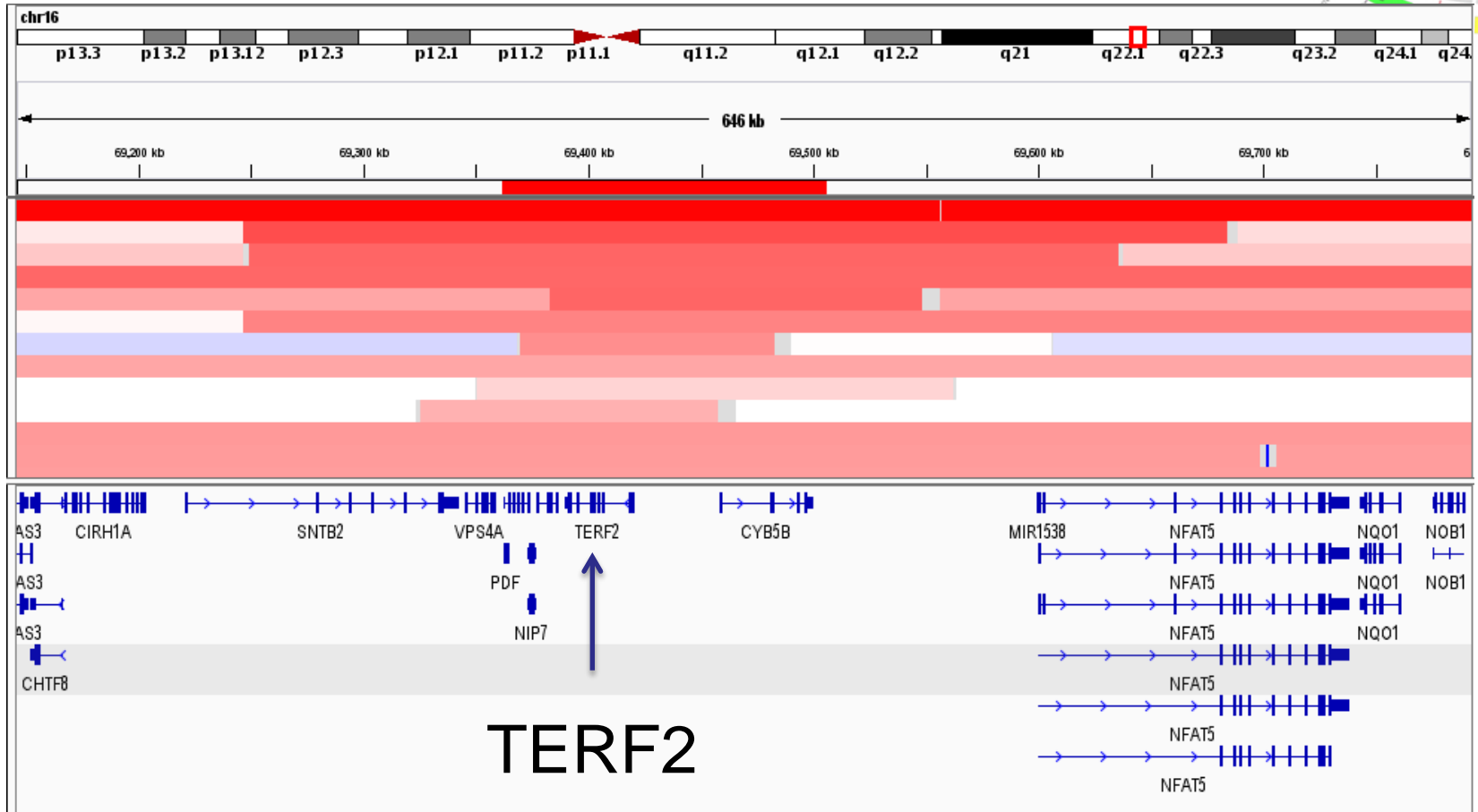
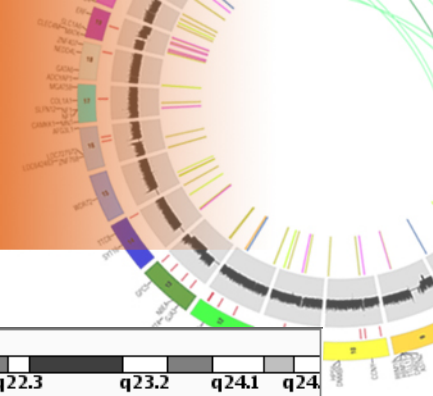
Significantly amplified and deleted DNA segments include important genes



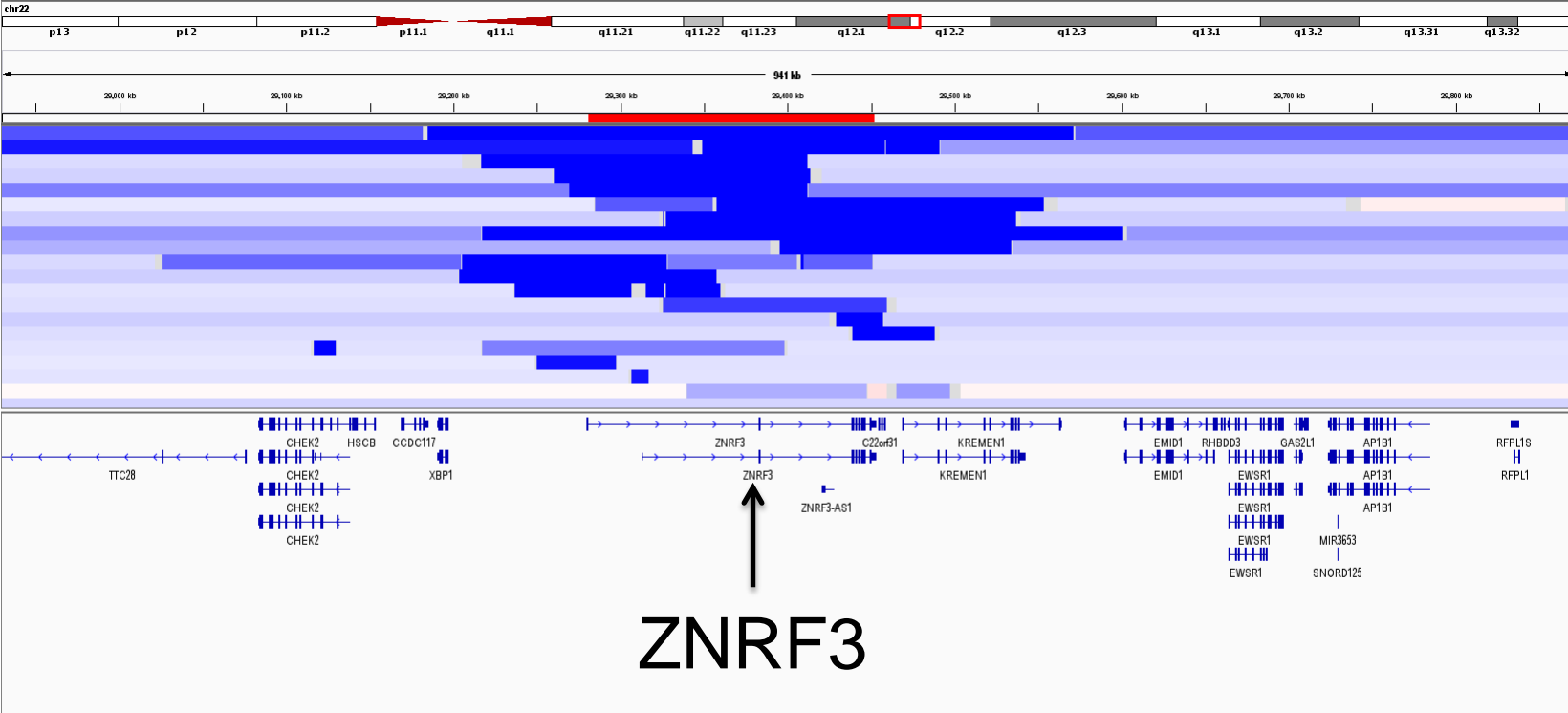
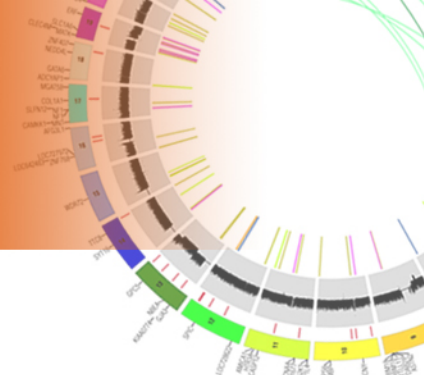
Andy Cherniack, Broad Institute



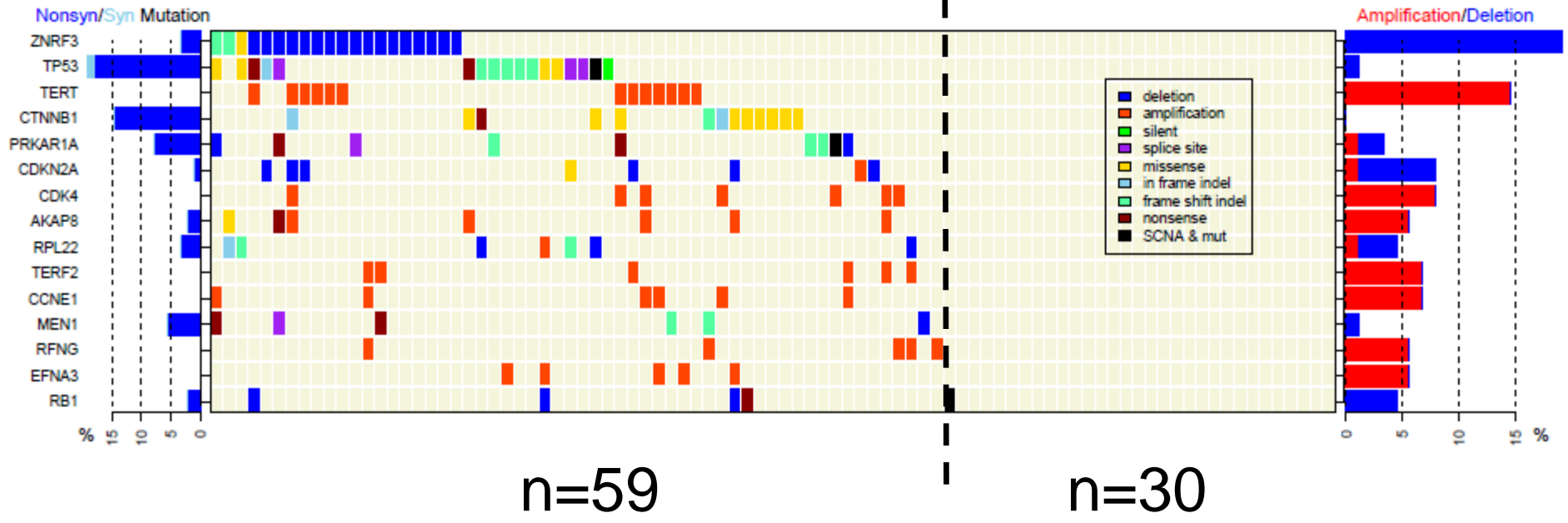
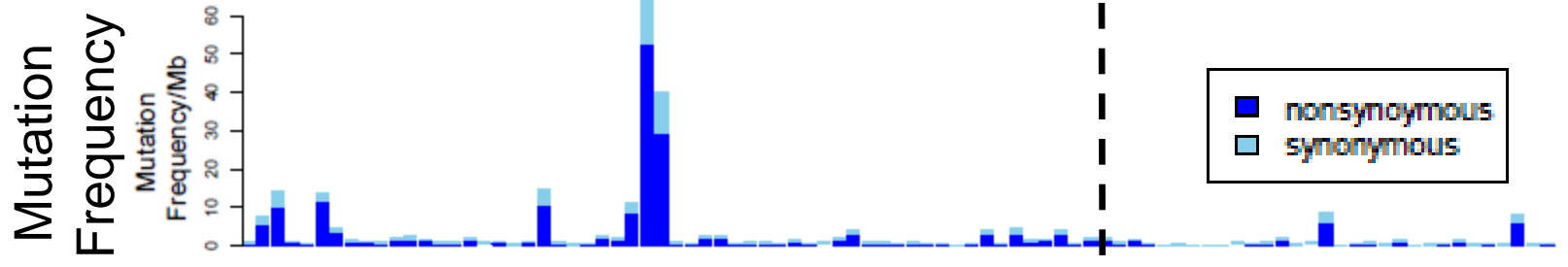
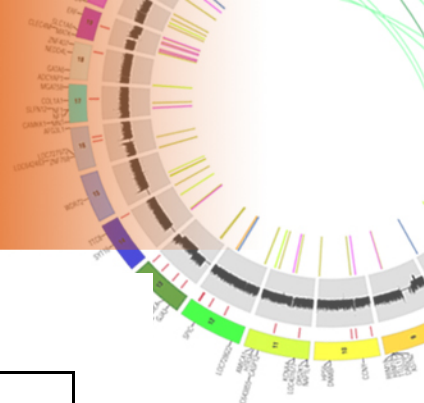
Novel TERF2 Focal Amplifications in 9 Tumors



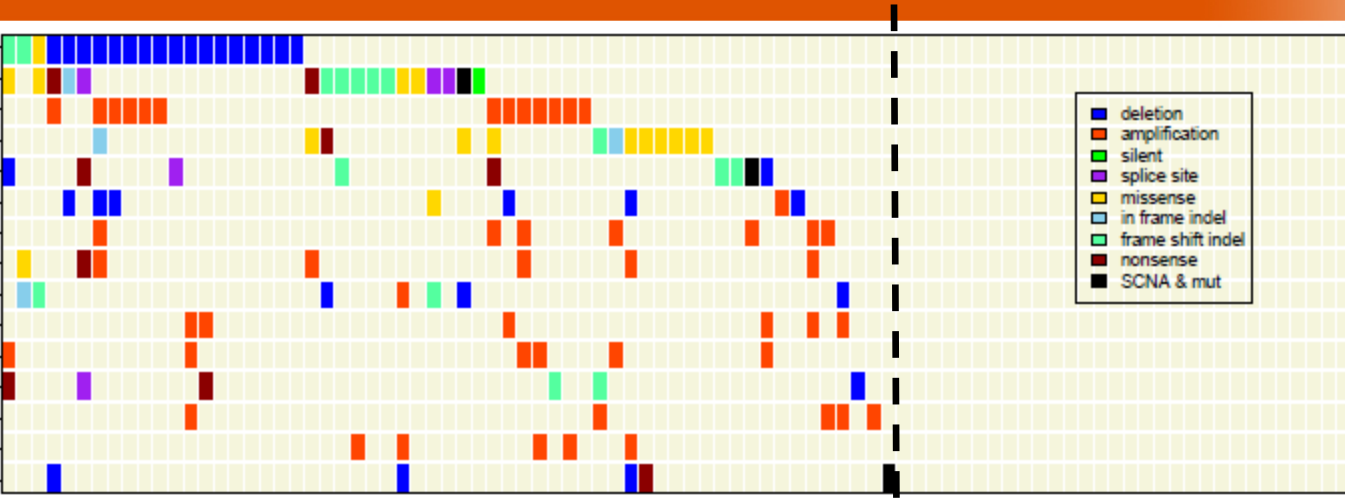
ZNRF3 Focal Deletions in 19 Tumors



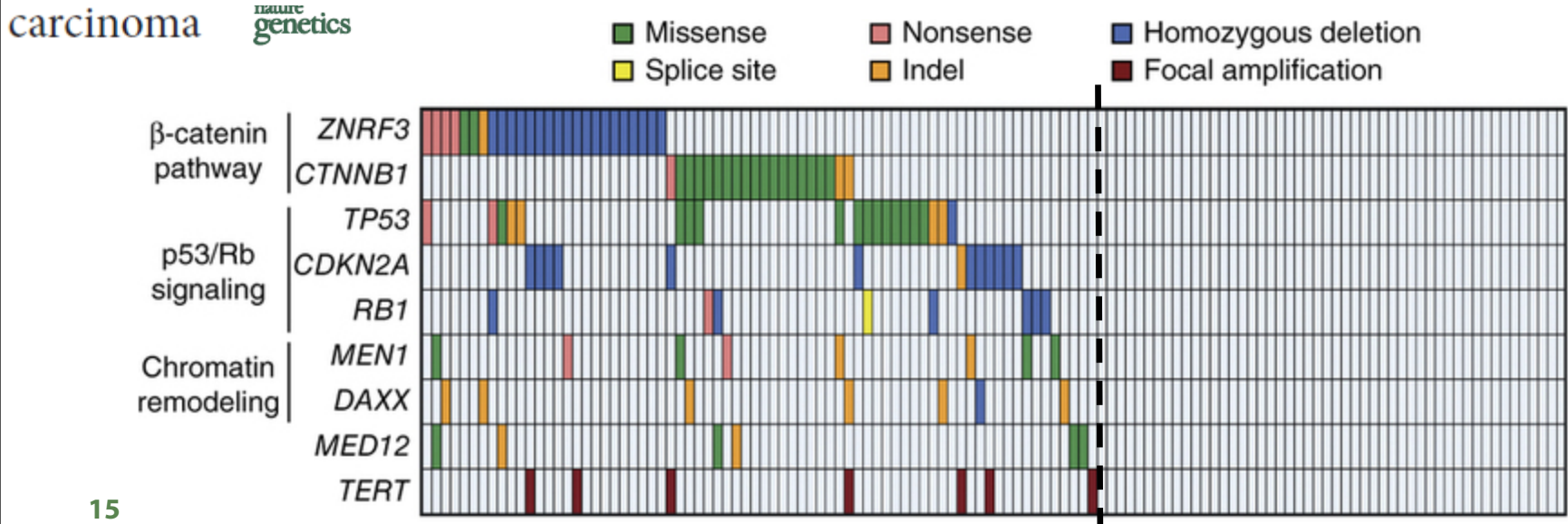
A subset of ACCs harbor no putative drivers



Recently published ENSAT paper shows very similar mutational landscape – exception for *PRKAR1A*

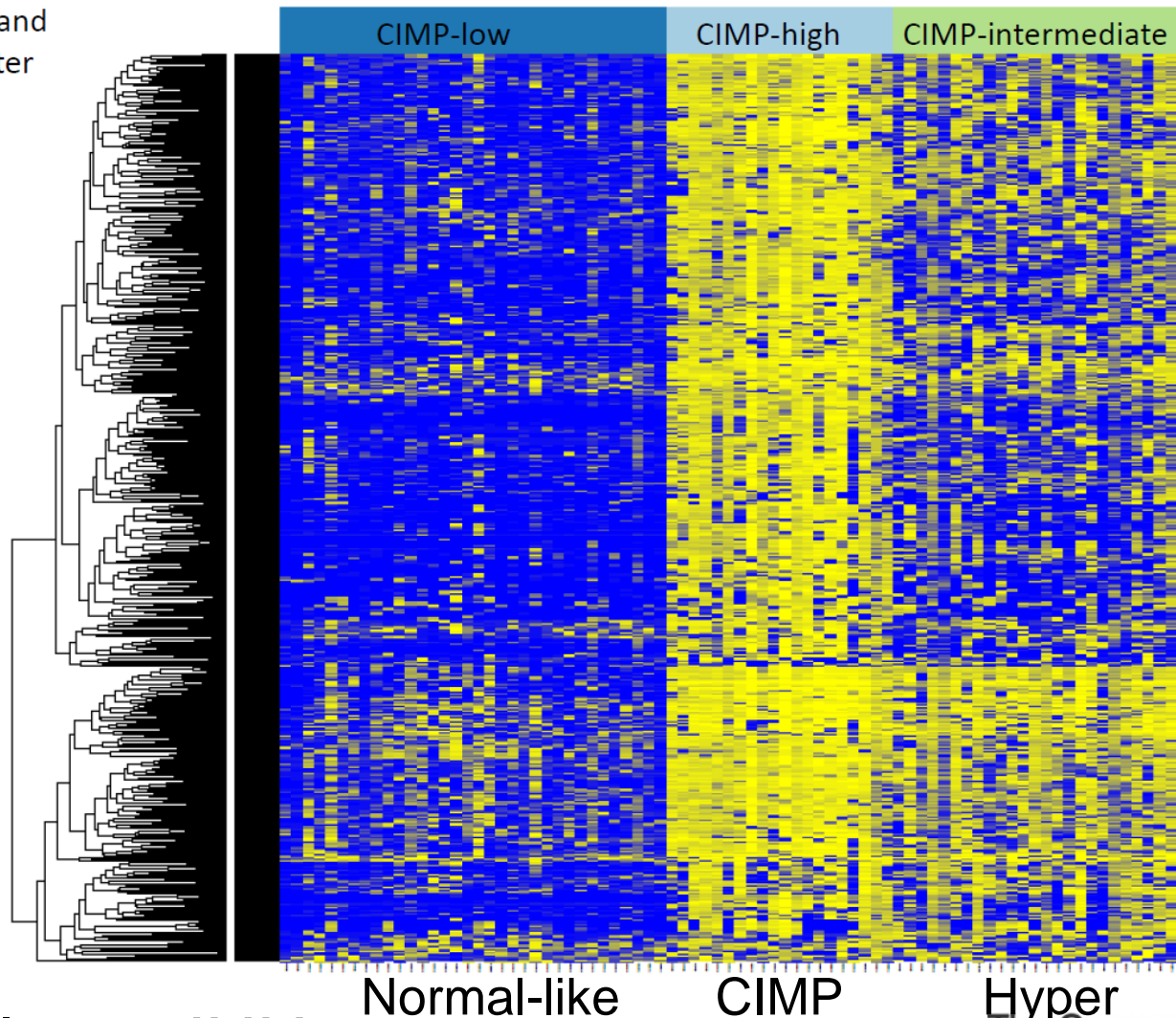


Integrated genomic characterization of adrenocortical carcinoma

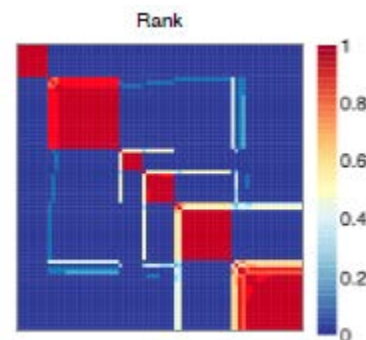
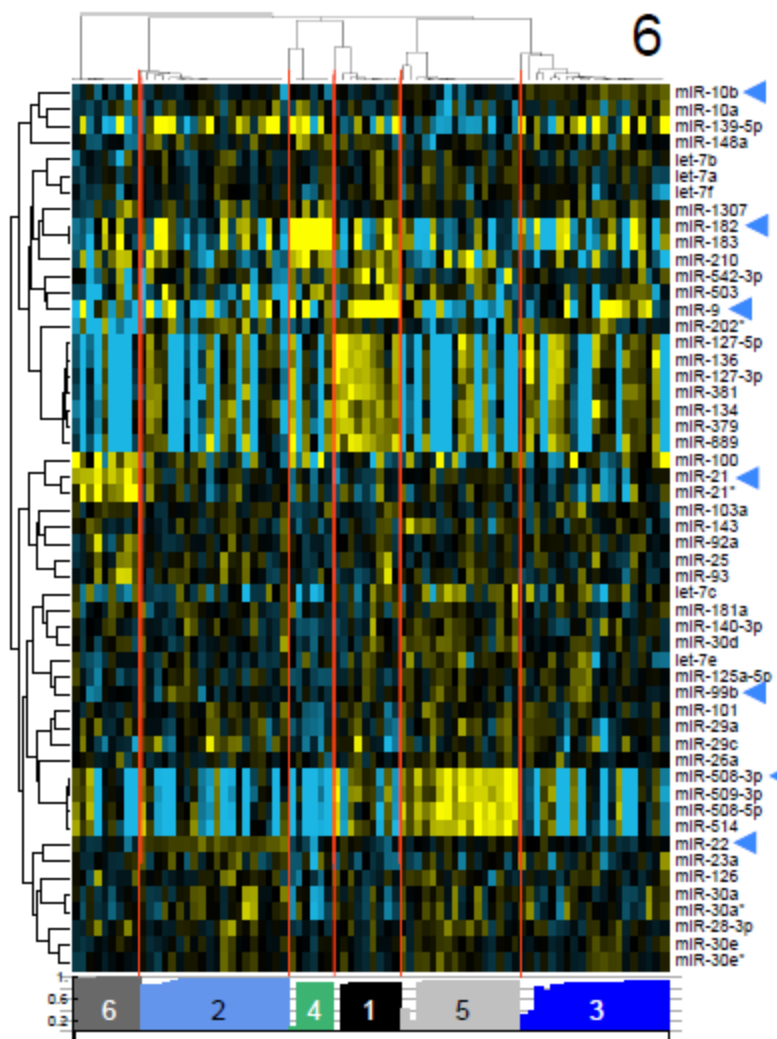


Methylation clustering found three groups with low-high methylation levels

CpG island promoter probes

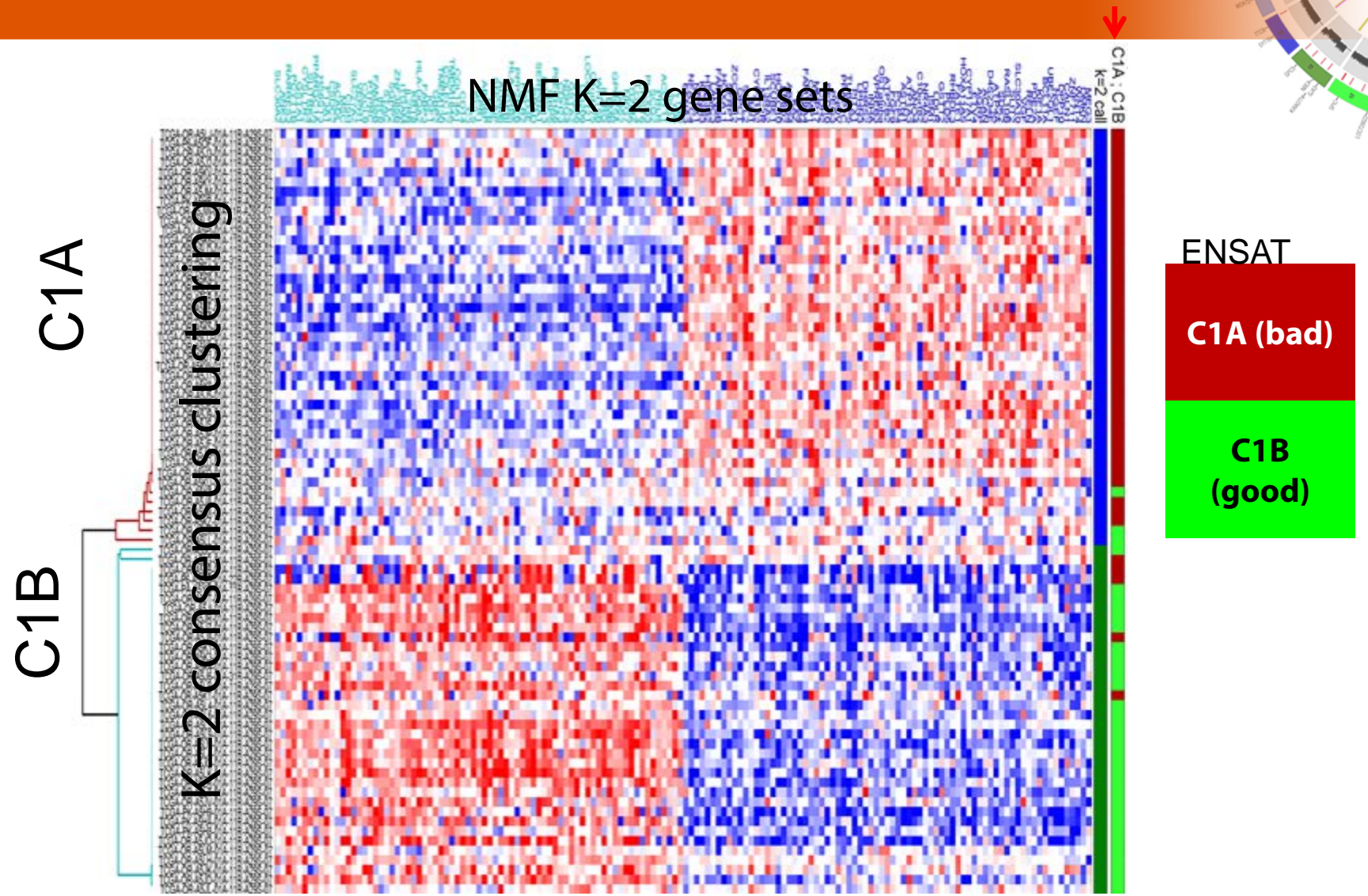


miRNA clustering found six clusters

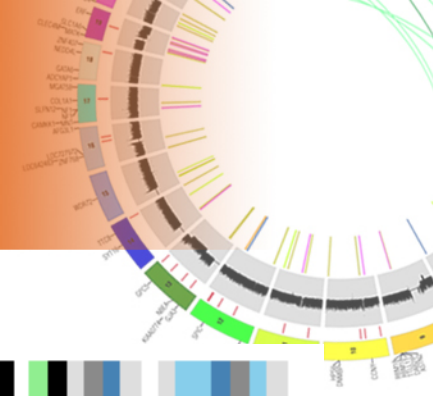


Gordon Robertson, BCGSC

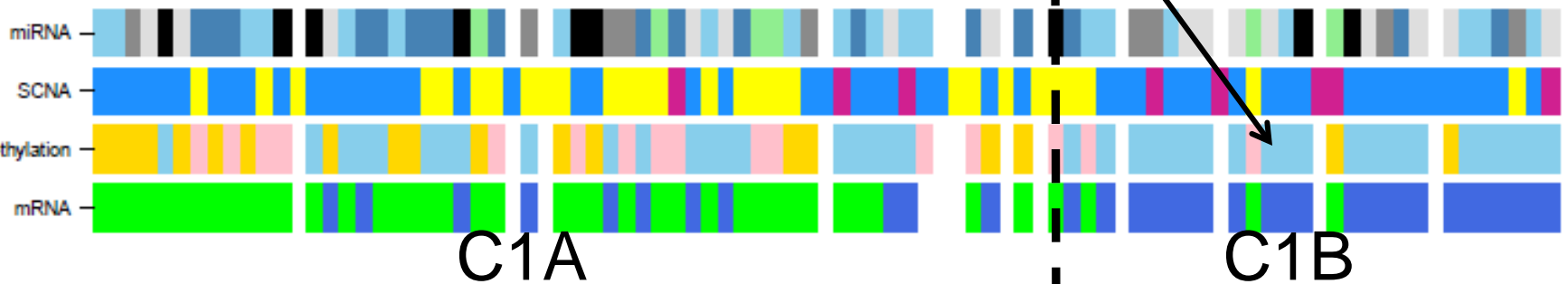
Gene expression clustering recapitulates ENSAT result



Genomic landscape of ACC is strongly reflected by subtypes

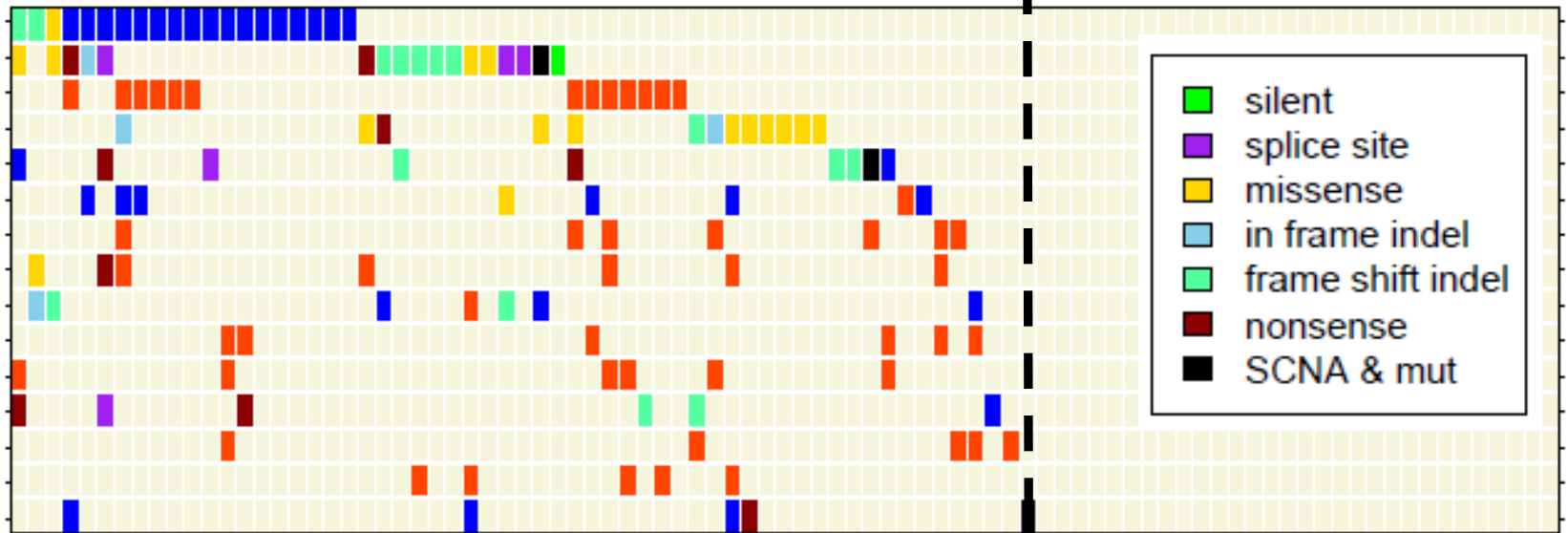


Normal-like



C1A

C1B



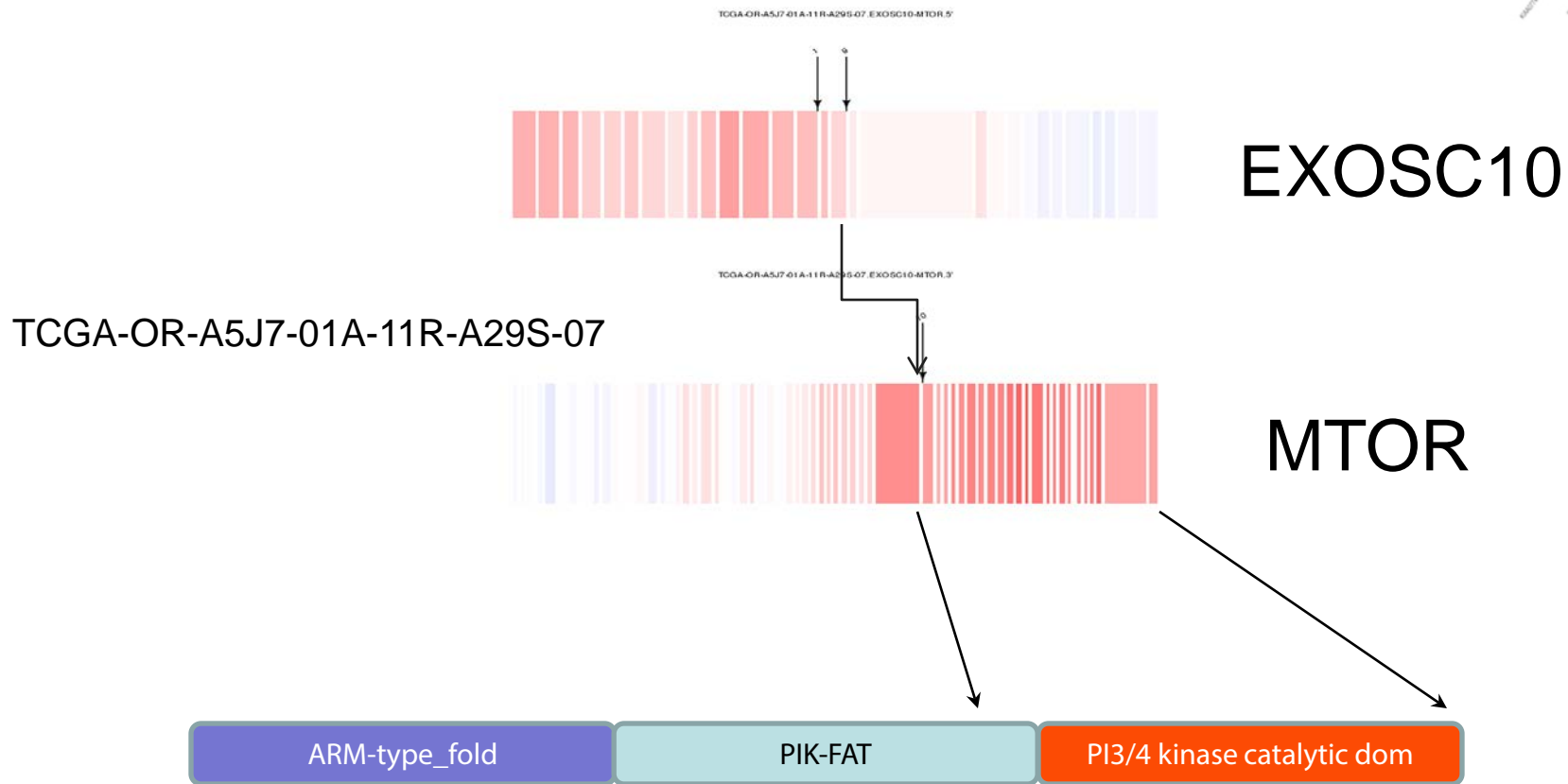
RNAseq data identifies putative cancer related gene fusions



5' Gene	Incidence	3' Gene	Frequency
FHOD3	2	NUSAP1	2
GANAB	2	MTOR	1
KDM6A	2	CD151	1
NSD1	2	BRE	1
PPP1CB	2	ESRRB	1
REPS2	2	RAD51C	1
TXNDC11	2		

- Fusion limited to those with breakpoints in coding region at both partner genes

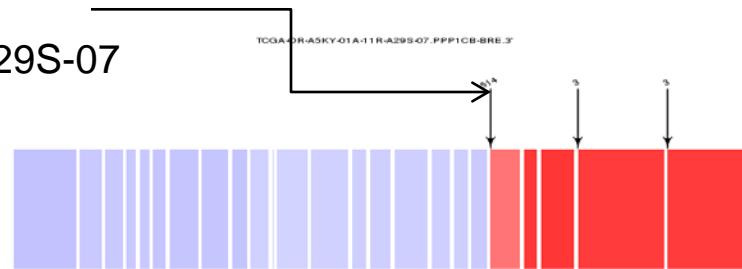
EXOSC10-MTOR fusion



PPP1CB-BRE fusion



PPP1CB



BRE

Biochemical and Biophysical Research Communications 326 (2005) 268–273

BRE enhances in vivo growth of tumor cells

ARTICLE

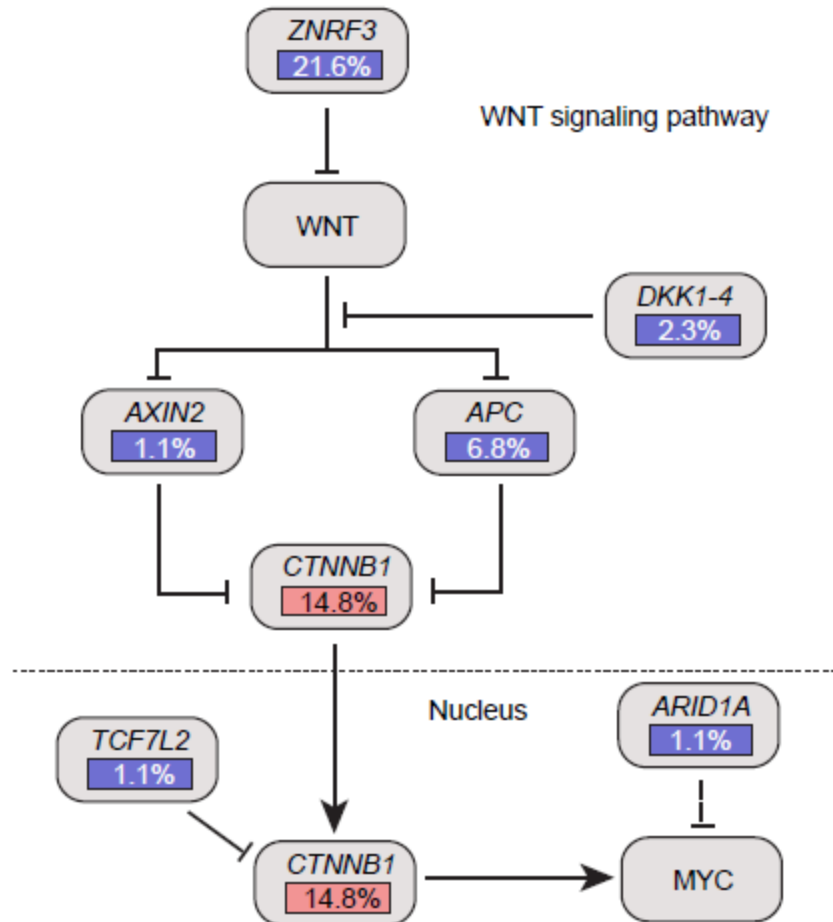
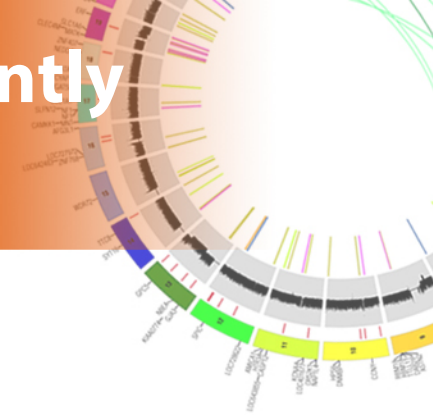
Volume 49(4): 491–499, 2001

The Journal of Histochemistry & Cytochemistry

<http://www.jhc.org>

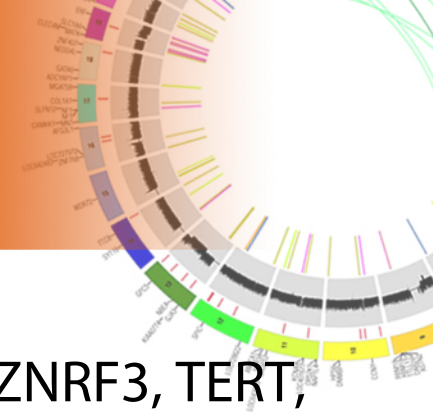
Differential Expression of a Stress-modulating Gene, BRE, in the Adrenal Gland, in Adrenal Neoplasia, and in Abnormal Adrenal Tissues

WNT signaling pathway is the most frequently altered pathway



Overall, 45.4% of the patients have at least one gene altered in WNT pathway

Summary



- We found potential new ACC driver genes, including ZNRF3, TERT, TERF2, PRKAR1A.
- We report characteristic mRNA/methylation/copy number/miRNA subtypes.
- Integrative analysis highlights ~30% of ACCs without an apparent driver alteration.
- Infrequent alterations, such as sporadic gene fusions, may contribute to adrenal tumorigenesis.
- WNT pathway is the most altered pathway in ACC, mostly by ZNRF3 deletion and CTNNB1 activating mutation.

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TCGA ACC Working Group

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Martin Fassnacht
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Jens Waldmann

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Jerome Bertherat
Guillaume Assie

Australia

Anthony Gill

ISB

Ilya Shmulevich
Theo Knijnenburg

Ontario

Sylvia Asa

MSKCC

Giovanni Ciriello

Support Team

Ina Felau
Margi Sheth
Yunhu Wan

