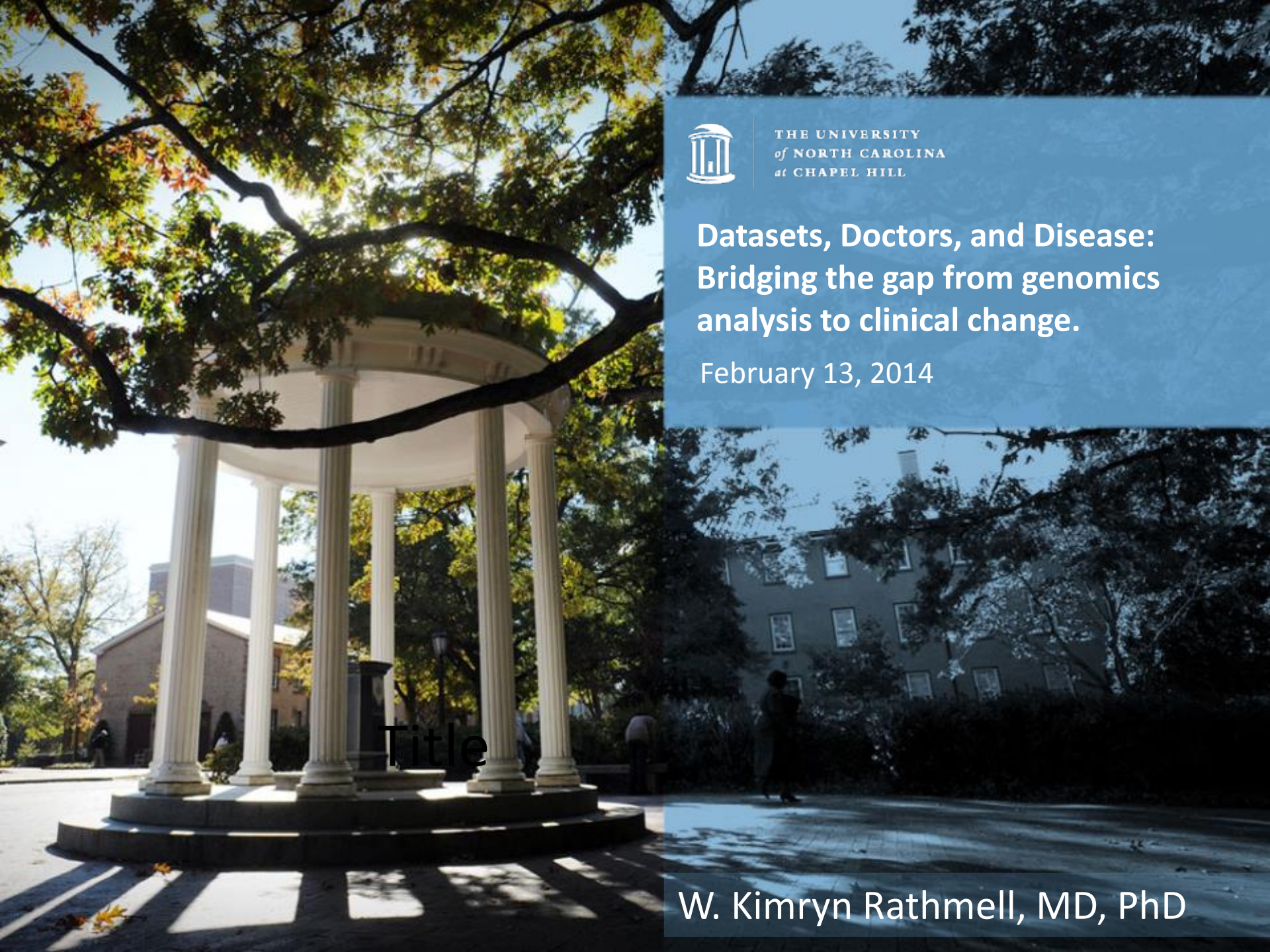
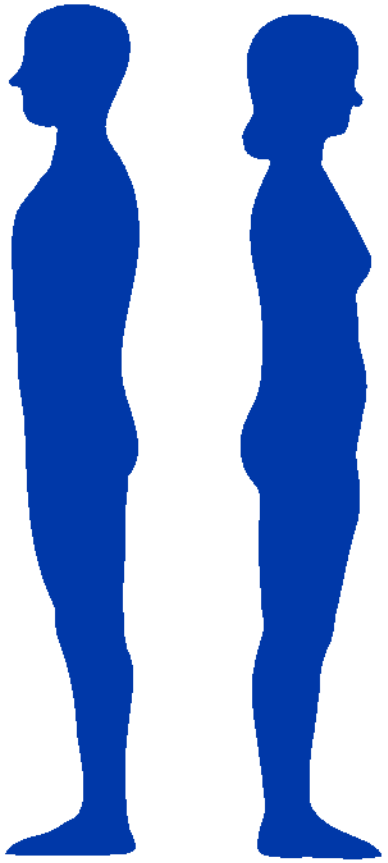

THE UNIVERSITY
of NORTH CAROLINA
at CHAPEL HILL
Datasets, Doctors, and Disease: Bridging the gap from genomics analysis to clinical change.
February 13, 2014
Title
W. Kimryn Rathmell, MD, PhD

Estimated New Cancer Cases* in the US in 2013

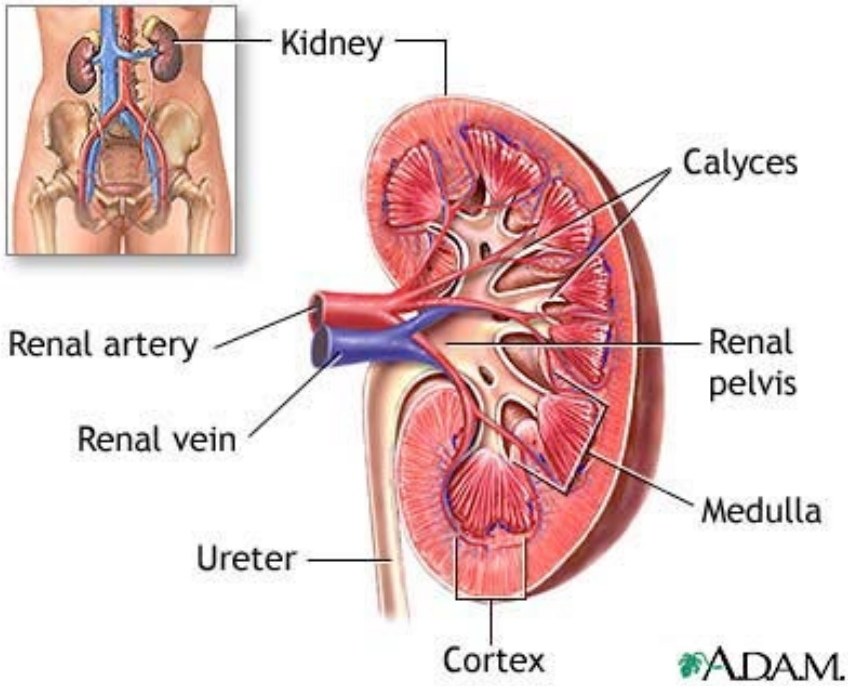
Men 854,790		Women 805,500	
Prostate	28%	29%	Breast
Lung & bronchus	14%	14%	Lung & bronchus
Colon & rectum	9%	9%	Colon & rectum
Urinary bladder	6%	6%	Uterine corpus
Melanoma of skin	5%	6%	Thyroid
Kidney & renal pelvis	5%	4%	Non-Hodgkin lymphoma
Non-Hodgkin lymphoma	4%	4%	Melanoma of skin
Oral cavity	3%	3%	Kidney & renal pelvis
Leukemia	3%	3%	Pancreas
Pancreas	3%	3%	Ovary
All Other Sites	20%	19%	All Other Sites



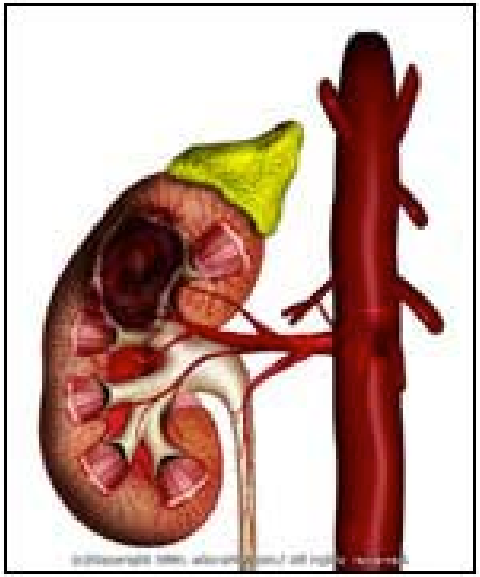
*Excludes basal cell and squamous cell skin cancers and in situ carcinoma except urinary bladder.

Renal Cell Carcinoma (RCC)

- Originates in the renal cortex
- Most common solid lesion occurring in the kidney (80-85% of all primary renal neoplasms)



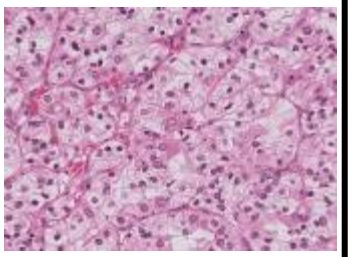
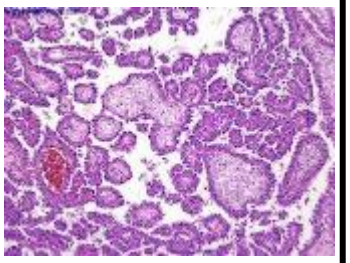
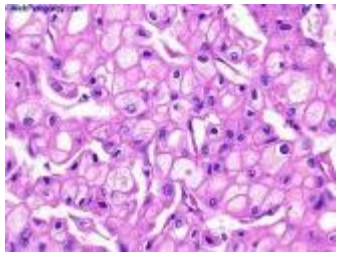
Diseased Kidney



Outline

- Appreciating differences in similar tumors.
- Using biological signatures to improve prognosis.
- The problem/opportunity of heterogeneity.
- Integrating epigenetic programs into the clinical and biological picture.

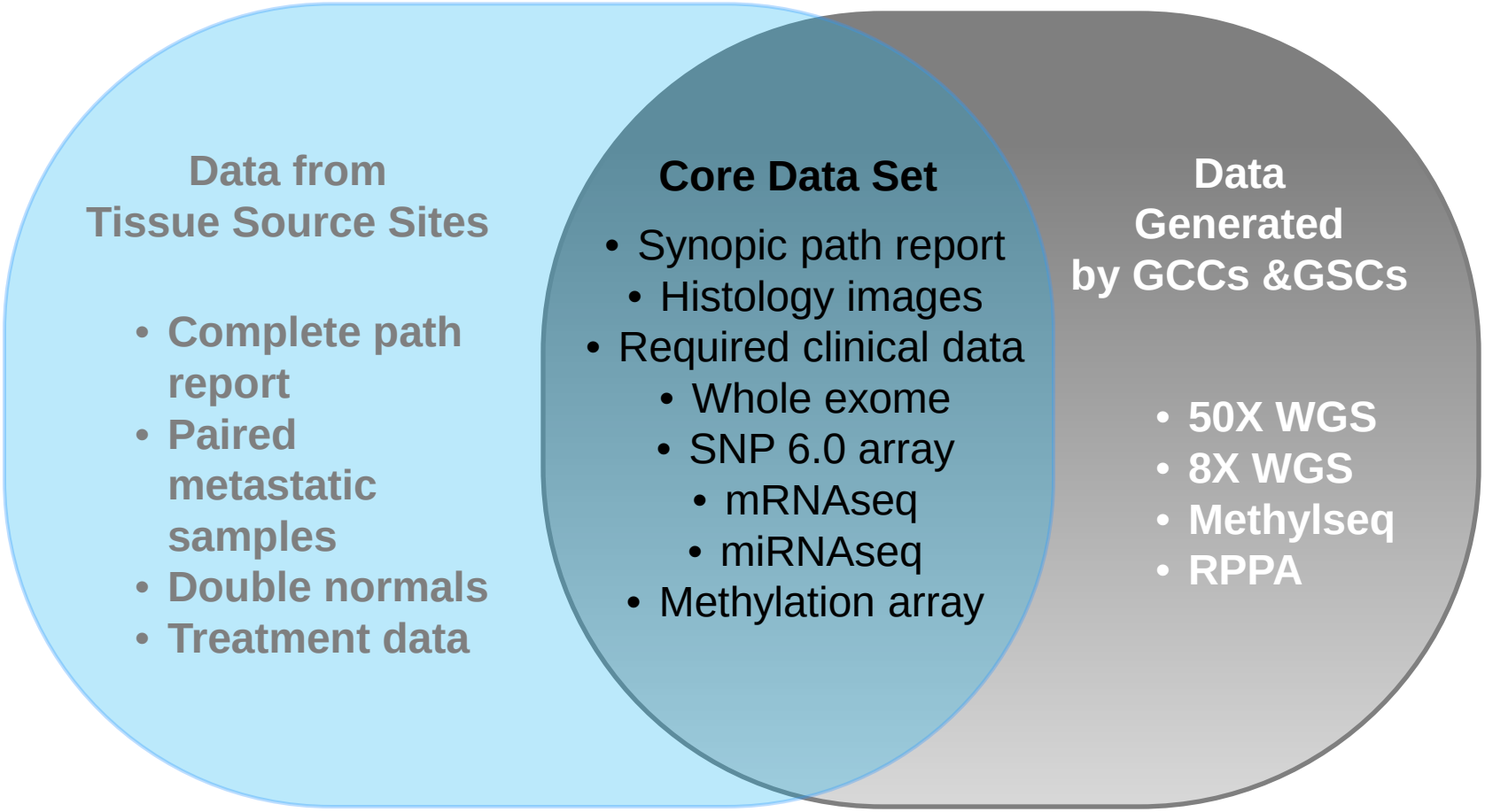
Renal Cell Carcinoma—not one disease

Subtype	Prevalence	Tumor Features	Microscopic Features
Clear cell carcinoma ccRCC	75–85%	Multinodular; large clear cells with prominent nucleoli, organized in nests surrounded by vessel bundles	
Chromophilic (papillary) carcinomas pRCC	10–15%	Ball-shaped outline, trabecular pattern, foamy macrophages, commonly multifocal	
Chromophobic carcinomas chRCC	5–10%	Bland nucleus, eosinophilic cytoplasm with central clearing.	

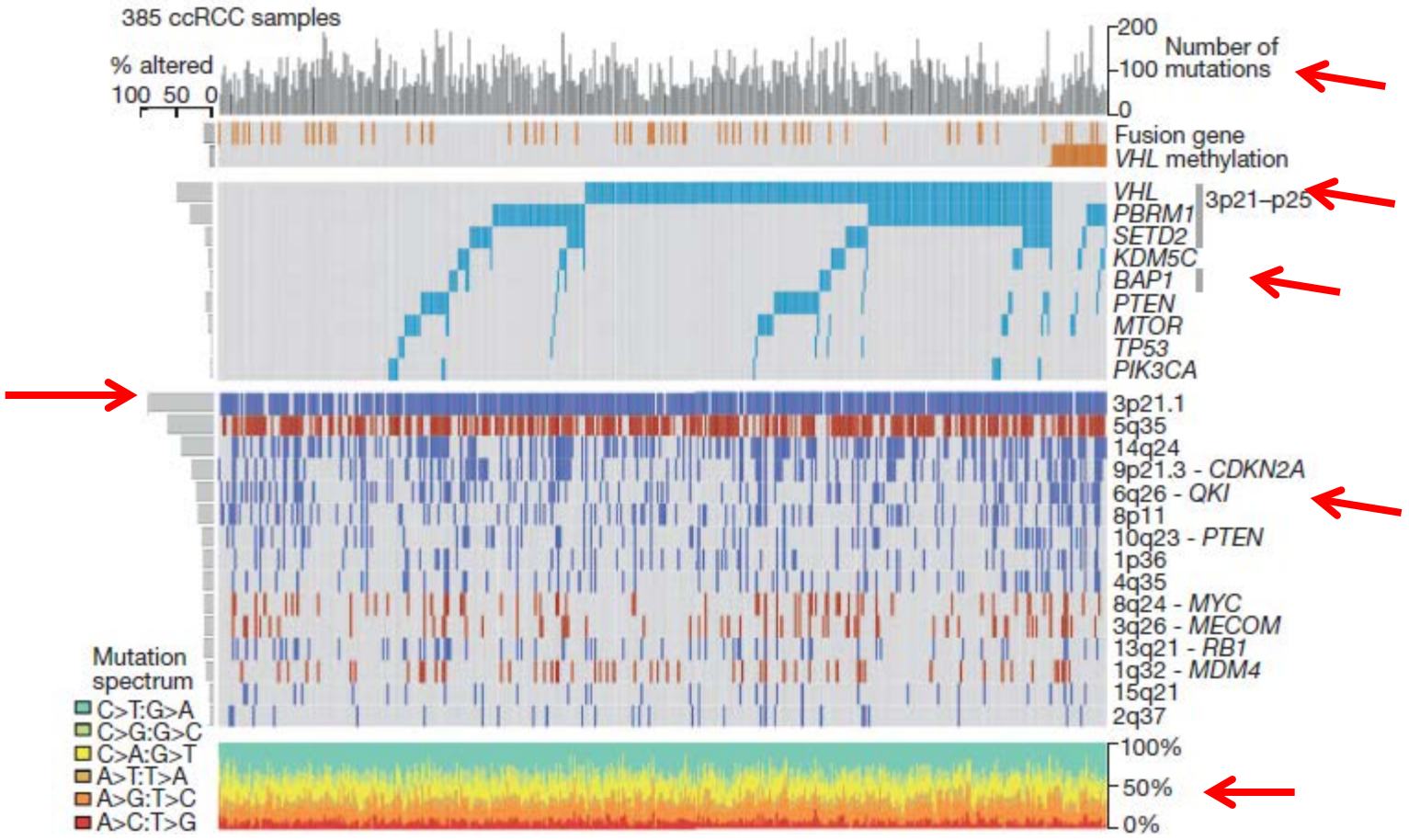
KIRC, KICH, KIRP

A tale of three kidney cancer genomes

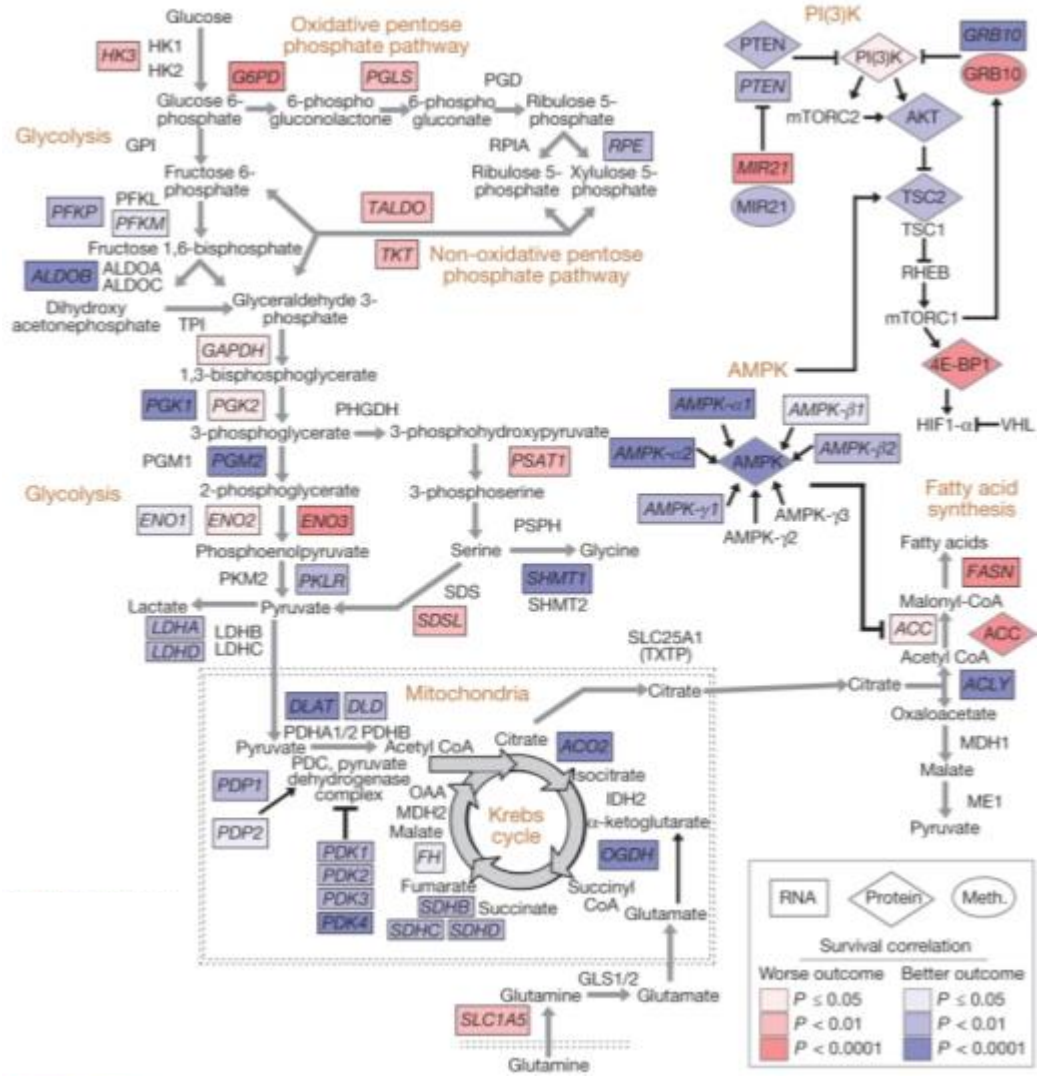
TCGA: What's in a Core Data Set?



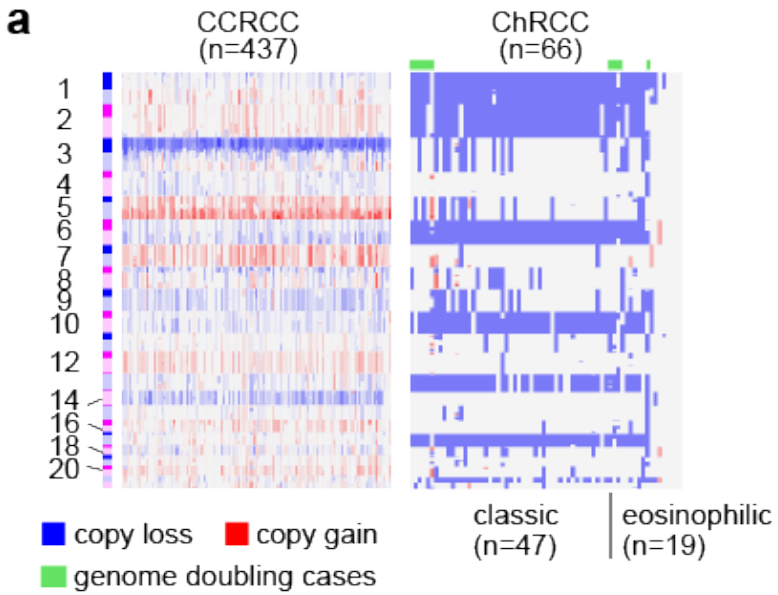
Meet ccRCC:


385 ccRCC samples
% altered
100 50 0
200
Number of
100 mutations
0
Fusion gene
VHL methylation
VHL
PBRM1
SETD2
KDM5C
BAP1
PTEN
MTOR
TP53
PIK3CA
3p21–p25
3p21.1
5q35
14q24
9p21.3 - CDKN2A
6q26 - QKI
8p11
10q23 - PTEN
1p36
4q35
8q24 - MYC
3q26 - MECOM
13q21 - RB1
1q32 - MDM4
15q21
2q37
Mutation
spectrum
C>T:G>A
C>G:G>C
C>A:G>T
A>T:T>A
A>G:T>C
A>C:T>G
100%
50%
0%

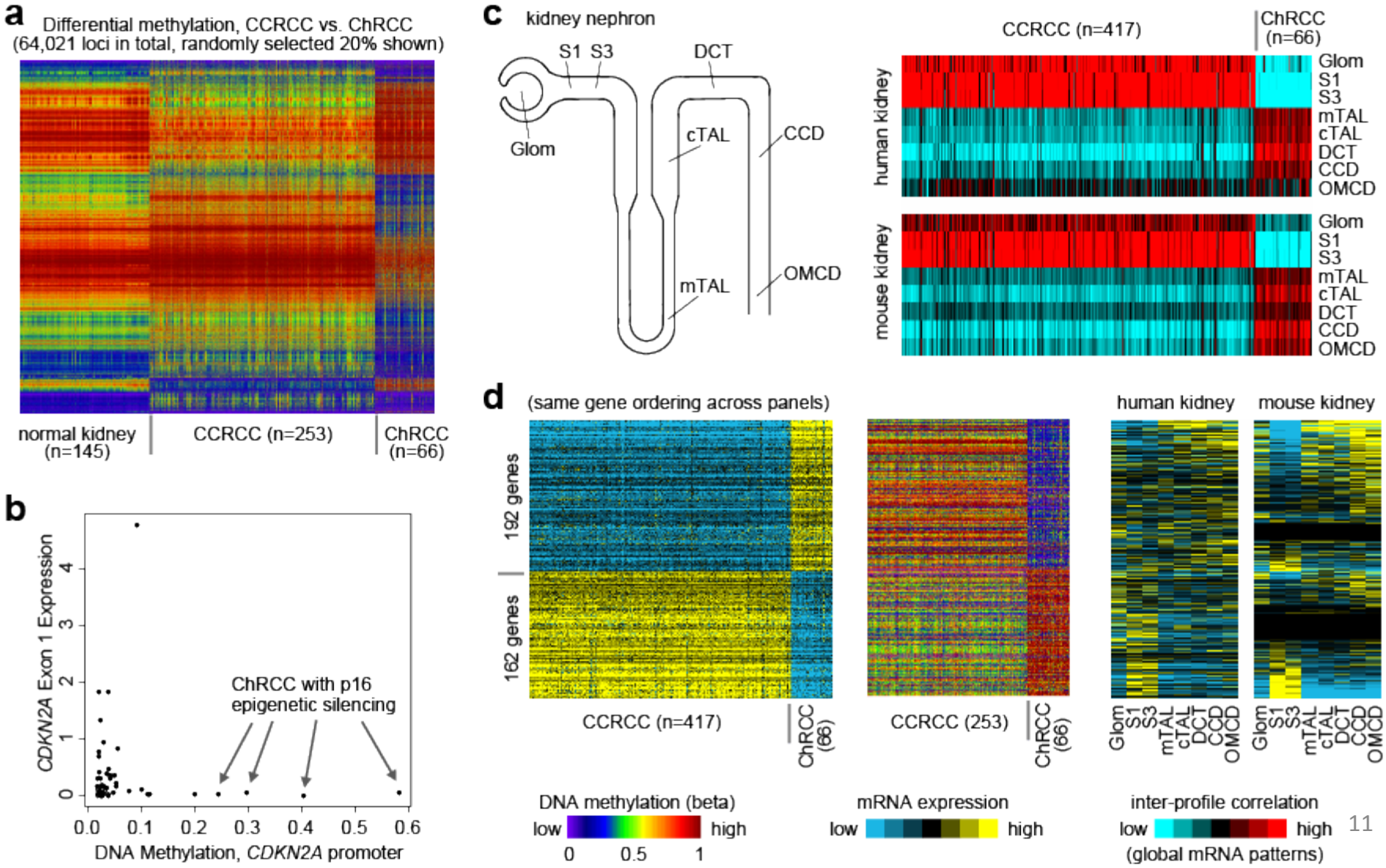
Metabolic Network: All glycolysis



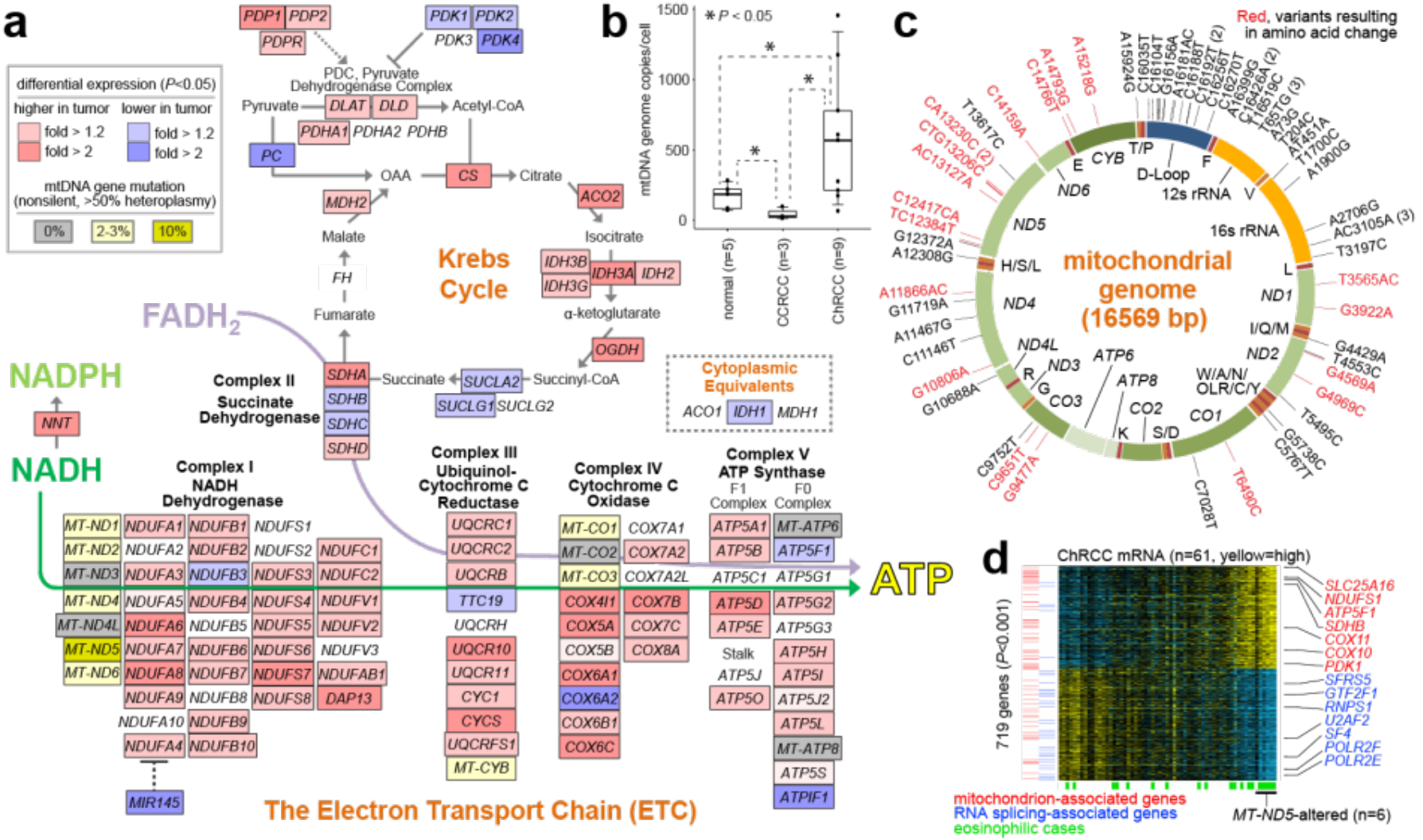
Now, meet Chromophobe RCC



Different methylation, expression, and origin in the nephron.

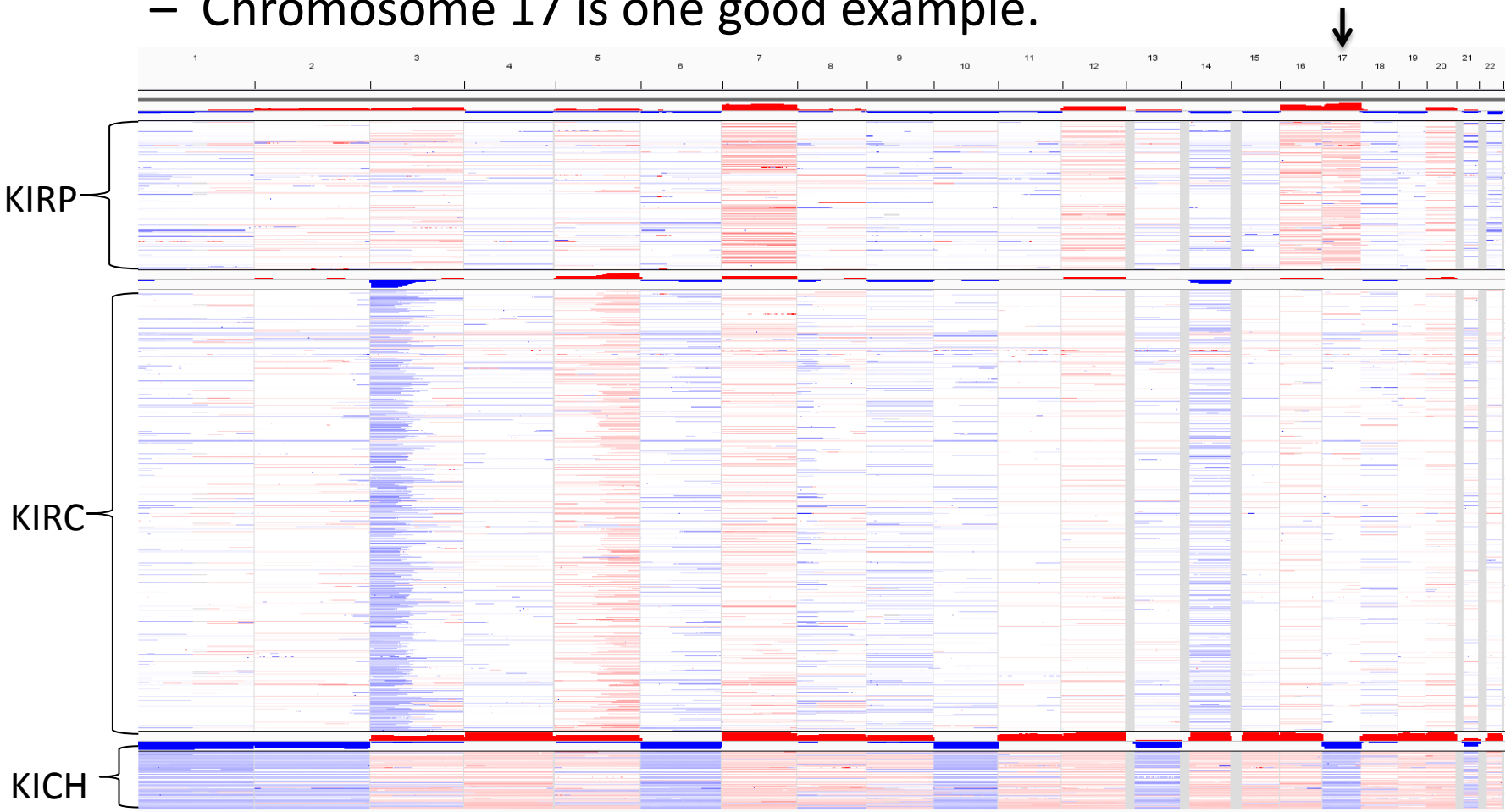


A different biology-focus on mitochondria



Comparing Copy Number Variation:

- KIRP, KICH, and KIRC display very different SCNA patterns
  - Chromosome 17 is one good example.



Summary

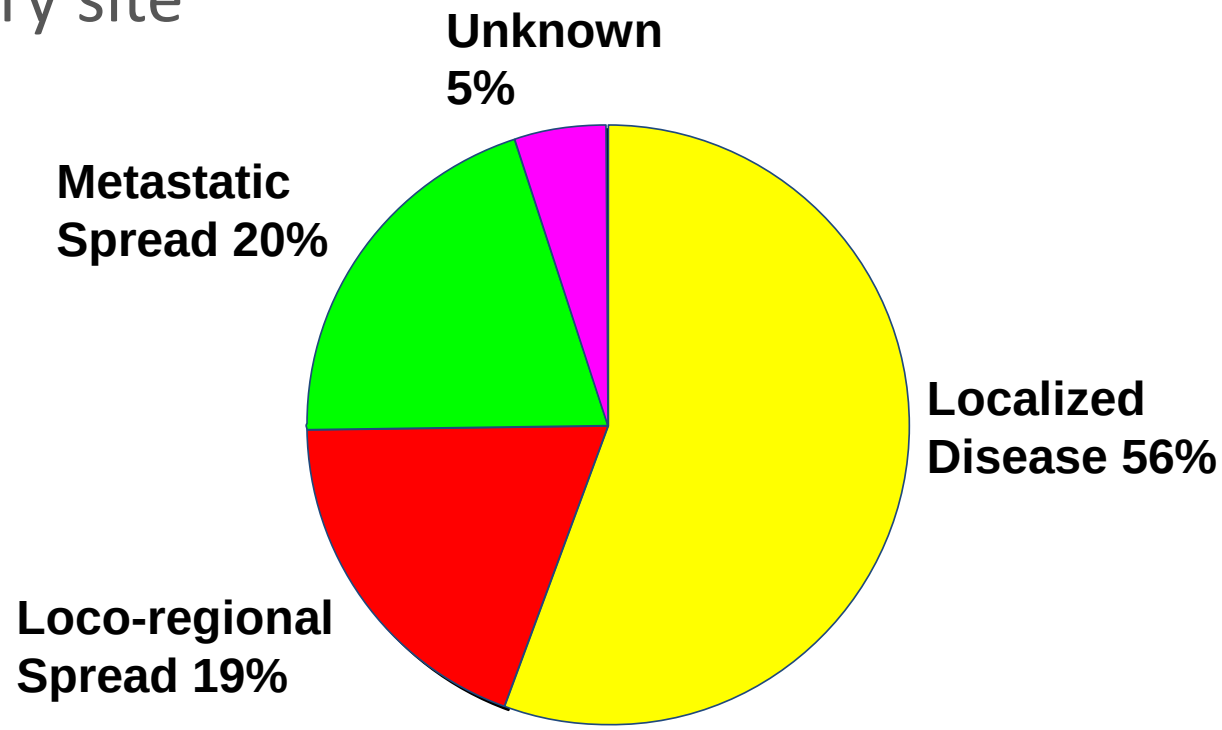
- The renal cell carcinomas represent highly distinct, *unrelated* diseases.
- The cancer genome atlas provides a framework for defining a cancer.

ccA + ccB = ccRCC

Molecular stratification of clear cell Renal Cell Carcinoma

Extent of Disease at Diagnosis

- Most cancers of the kidney and renal pelvis are diagnosed when the disease is still localized to the primary site

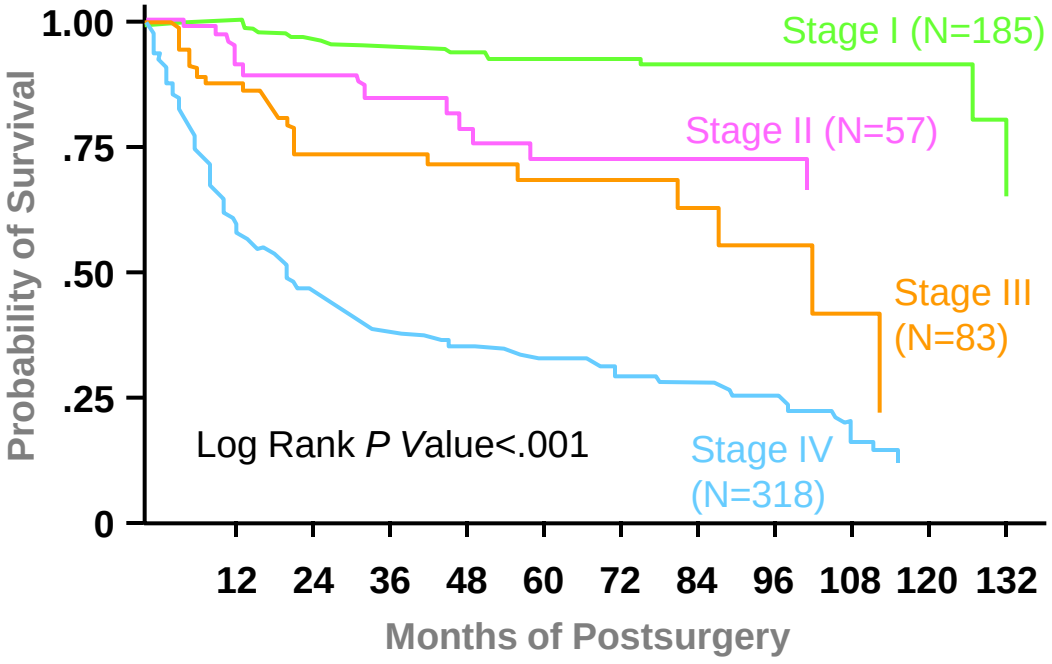


National Cancer Institute. SEER Stat Fact Sheets. Available at: http://seer.cancer.gov/statfacts/html/kidrp.html. Accessed August 28, 2008.

Determining Prognosis: Anatomic Extent of Disease

- Most consistent factor used to determine RCC prognosis

5-year Cancer-specific Survival Based on TNM Stage



TNM Stage	5-year Cancer-specific Survival
Stage I	91 ± 2.5%
Stage II	74 ± 6.9%
Stage III	67 ± 6.1%
Stage IV	32 ± 3.2%

Reprinted with permission from Tsui KH, et al. *J Urol*. 2000;163:1090-1095.

RCC Algorithms for cancer-specific survival

External Validation of the Mayo Clinic Stage, Size, Grade, and Necrosis (SSIGN) Score for Clear-Cell Renal Cell Carcinoma in a Single European Centre Applying Routine Pathology

Richard Zigeuner[a], Georg Hutterer[a], Thomas Chromecki[a], Arvin Imamovic[a], Karin Kampel-Kettner[a], Peter Rehak[b], Cord Langner[c], Karl Pummer[a]

Parameter	Score points
Pathologic tumour category	
pT1	0
pT2	1
pT3a	2
pT3b	2
pT3c	2
pT4	0
Nodal status	
Nx	0
pN0	0
pN1	2
pN2	2
Metastasis category	
M0	0
M1	4
Tumour size	
<5 cm	0
≥5 cm	2
Tumour grade	
1	0
2	0
3	1
4	3
Tumour necrosis	
Absent	0
Present	2

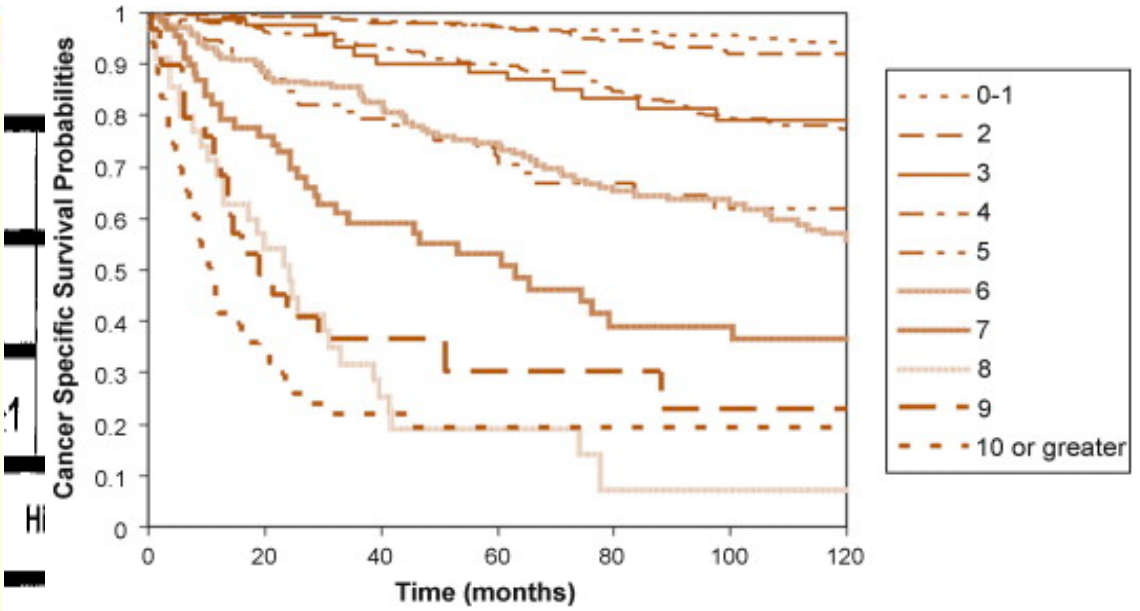
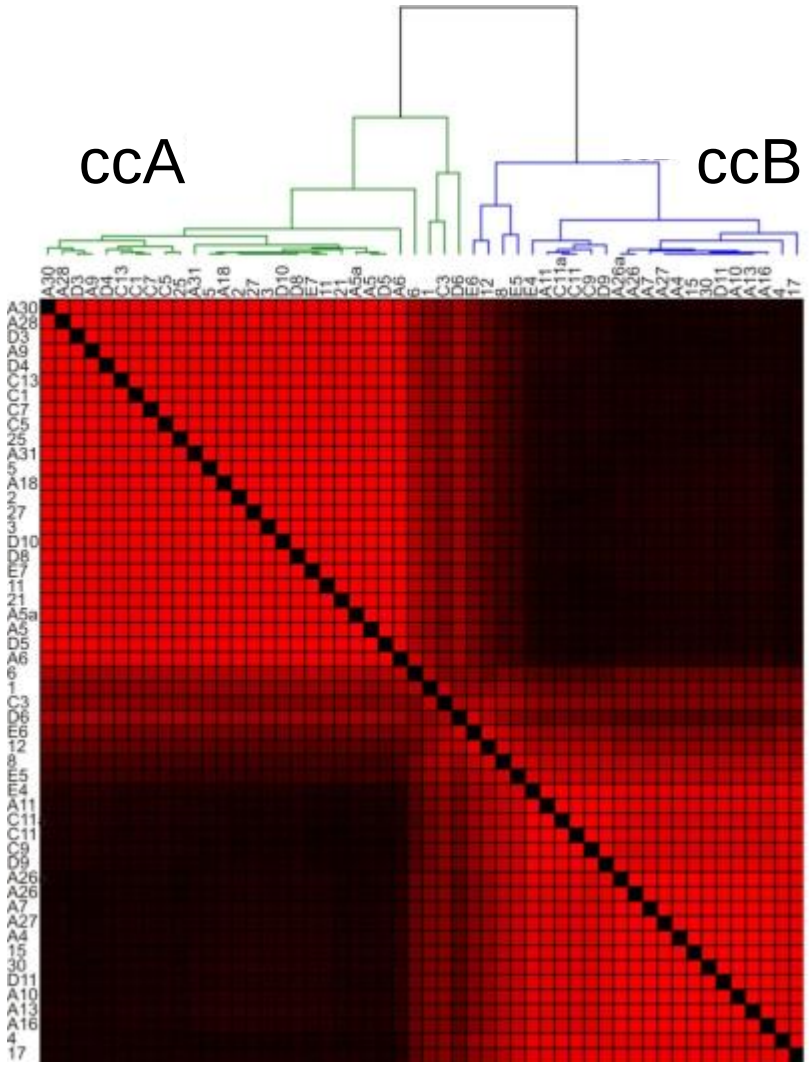


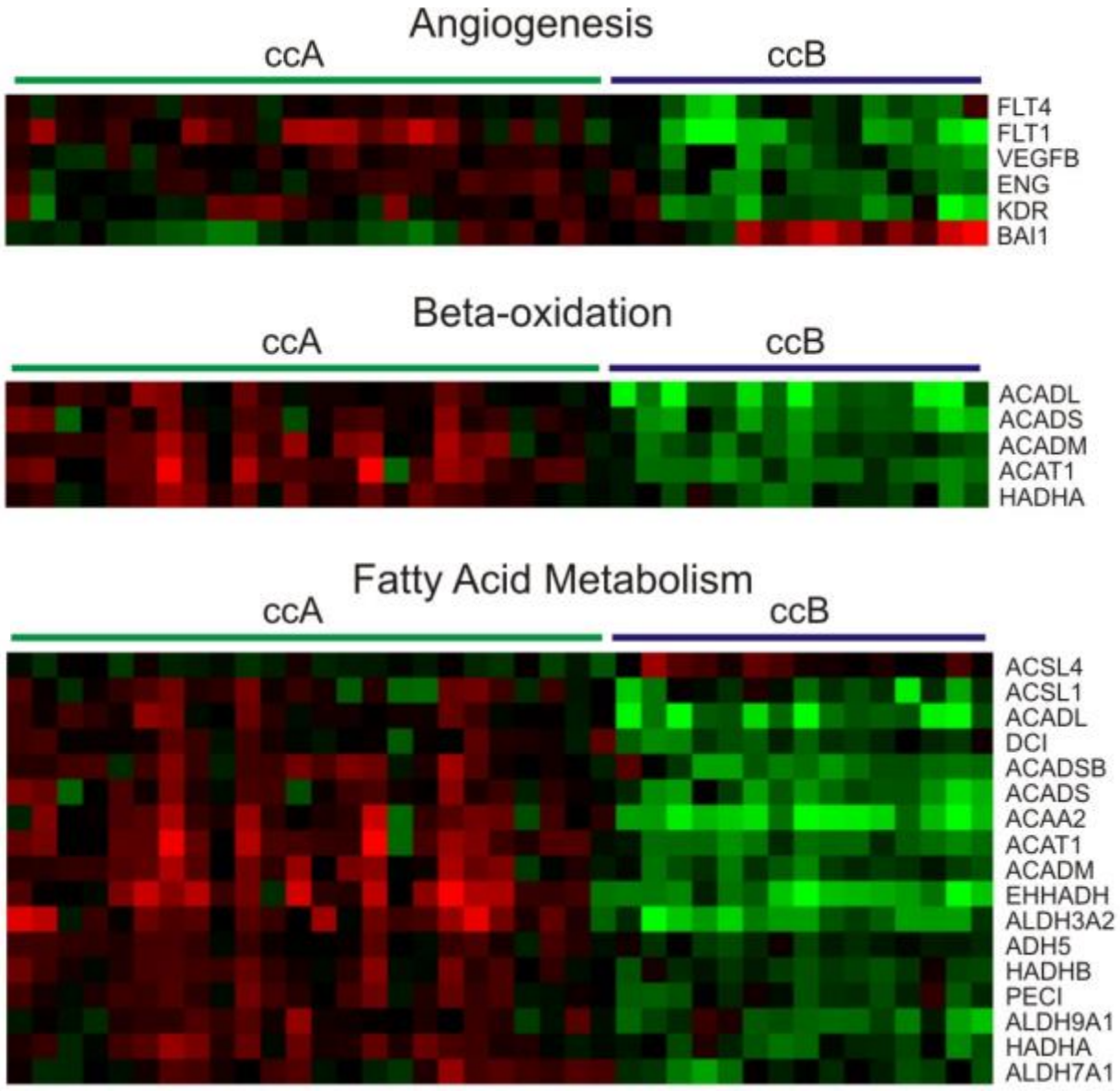
Fig. 1. Kaplan-Meier curves showing estimated 10-yr cancer-specific survival probabilities of the present study population related to respective SSIGN-score categories ($p < 0.001$, log-rank test).

Consensus clusters permit refined analysis



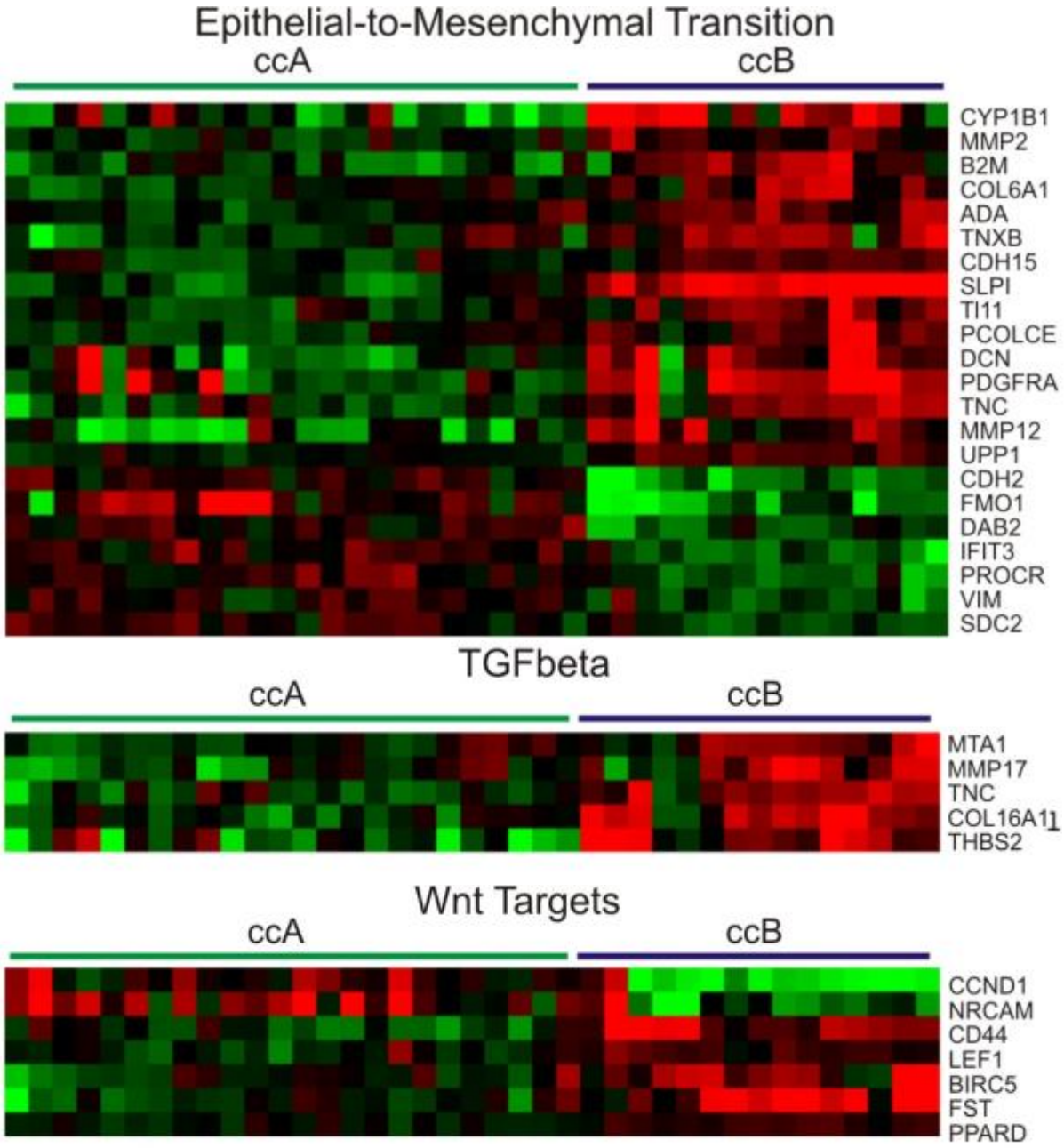
Brannon, et al, Genes and Cancer, 2010

ccA overexpresses RCC pathways



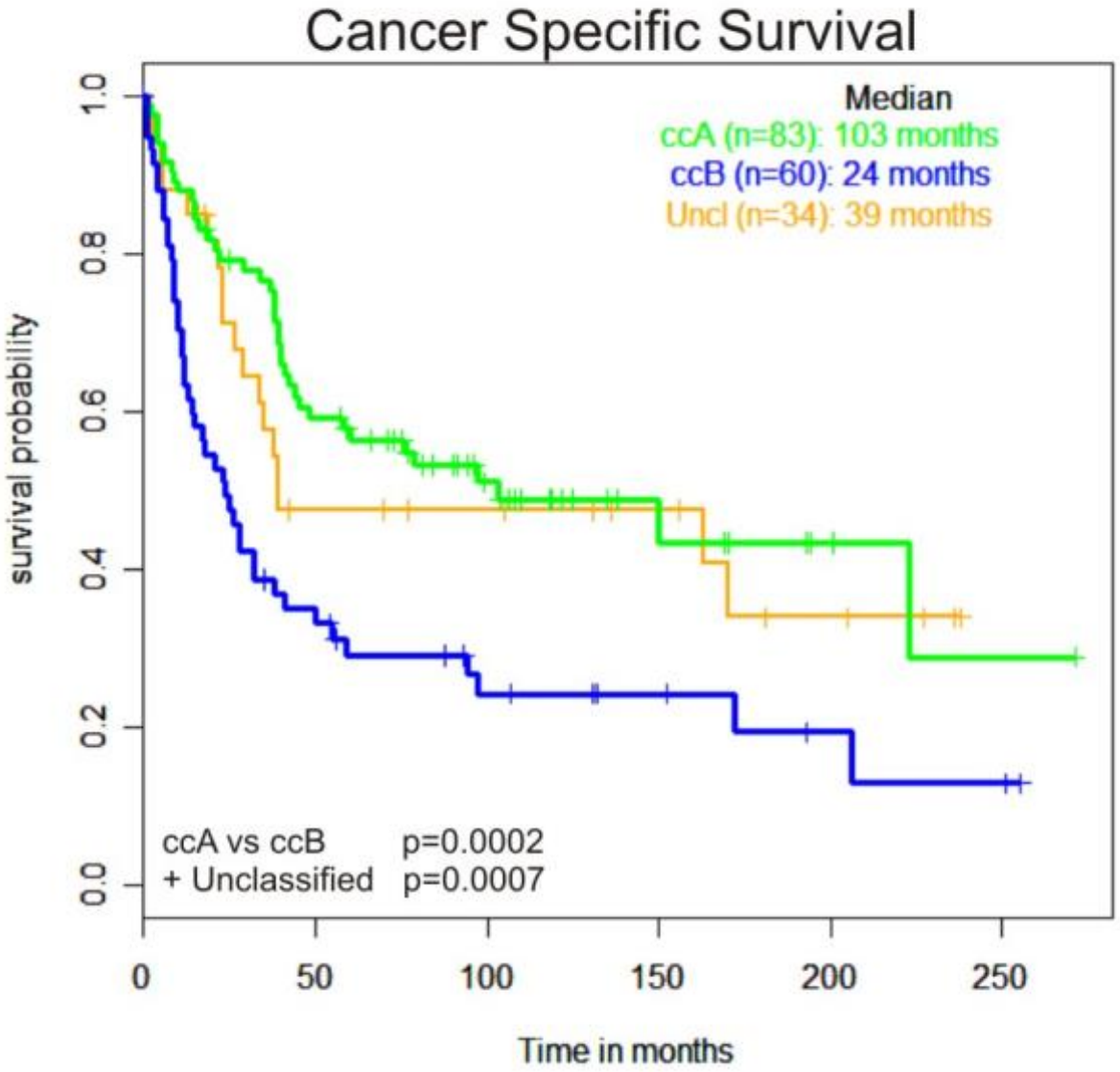
Brannon, et al, Genes and Cancer, 2010

ccB overexpresses aggressive genes



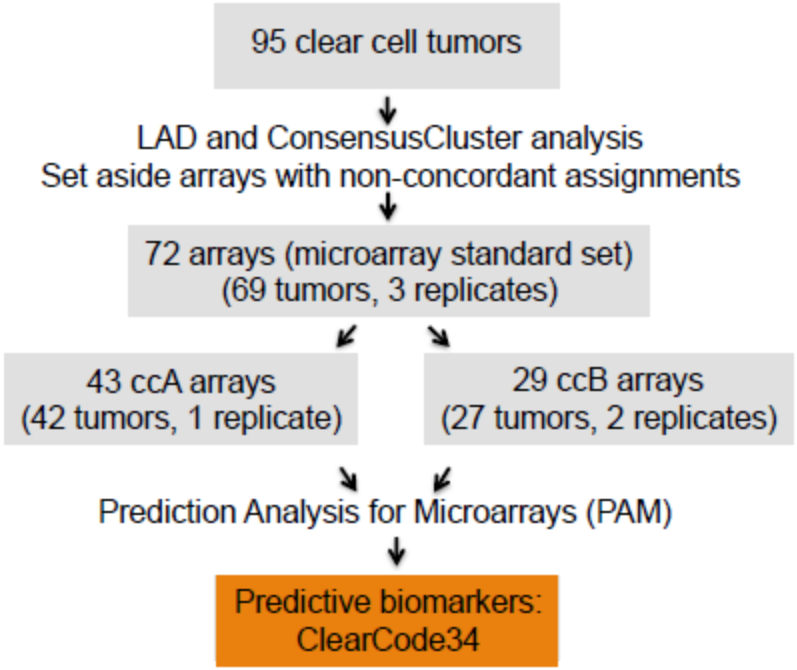
Brannon, et al, Genes and Cancer, 2010

Marked survival differences between subtypes in validation set

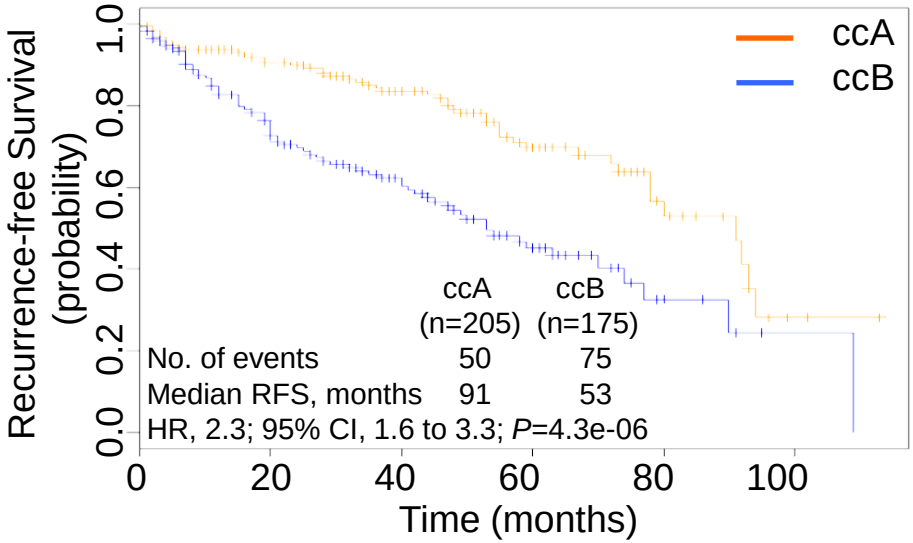


Brannon, et al, Genes and Cancer, 2010

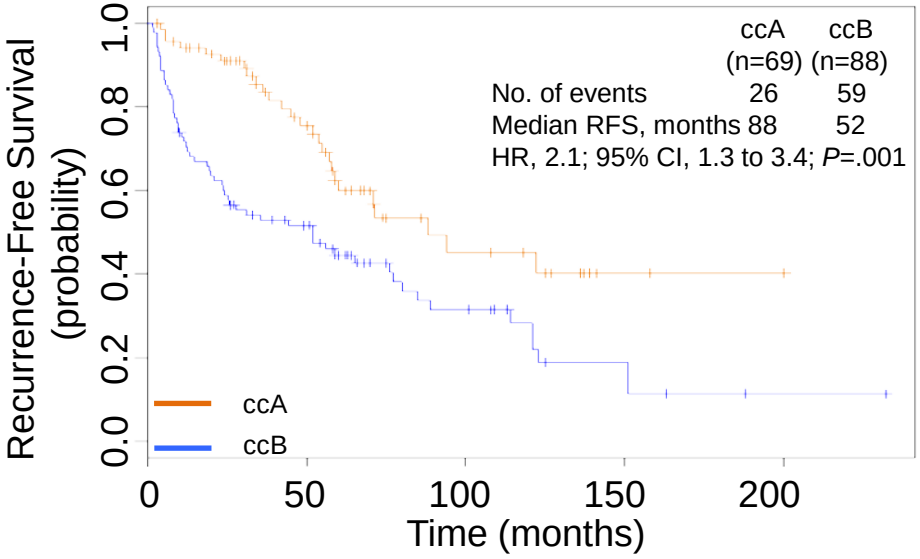
Creating a predictive tool



Prognostic value of ClearCode34 evaluated in TCGA



Prognostic value of ClearCode34 validated in UNC cohort



Prognostic value of ClearCode34 validated in TCGA

Variable	Univariate Model HR	Univariate Model *P*	Multivariate Model HR	Multivariate Model *P*	Final Model *P*
Subtype*	2.2	<.001	1.7	<.001	.0009
Stage$		<.001			.0007
II	1.4	.121	1.3	.307	
III/IV	2.4	<.001	1.8	<.001	
Grade||		<.001			<.0001
3	1.6	.002	1.3	.138	
4	5.1	<.001	3.1	<.001	

Abbreviation: HR, hazard ratio

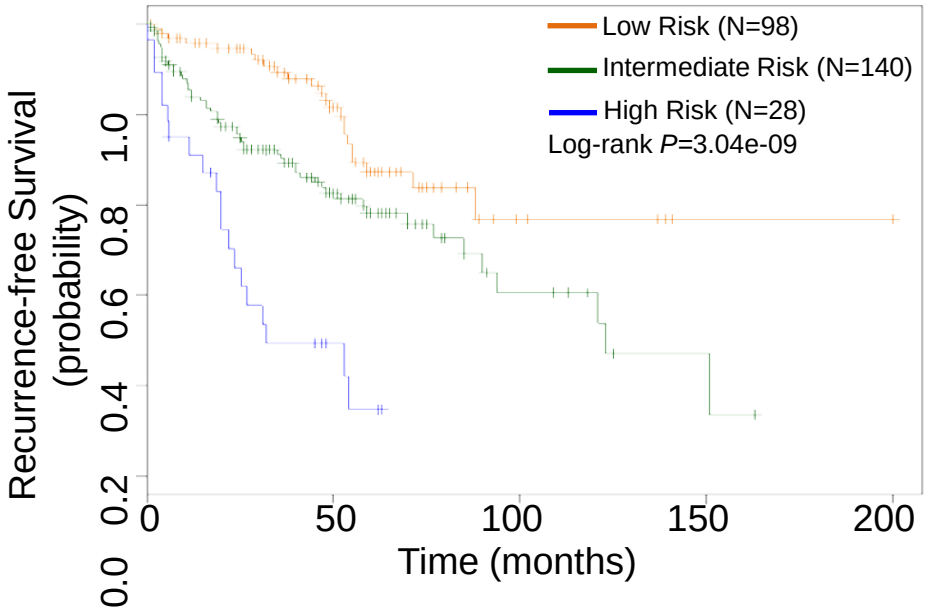
Subtype ccA was used as reference in univariate and multivariate analysis.

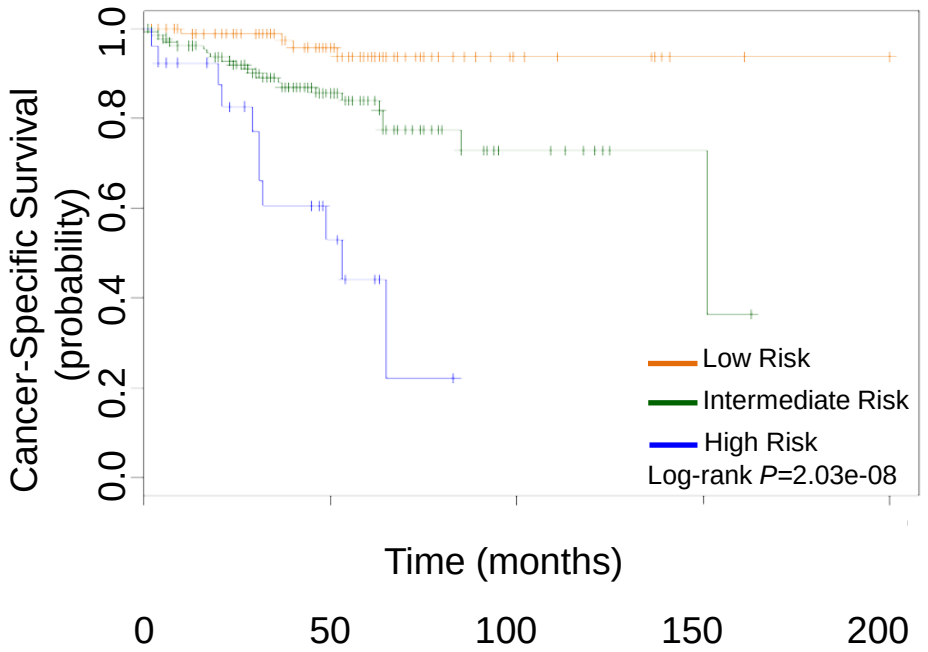
$ Stage I was used as reference in univariate and multivariate analysis. Stage was encoded as an ordinal variable with three levels.

|| Grade 1 and 2 were combined and used as reference in univariate and multivariate analysis. Grade was encoded as an ordinal variable with three levels.

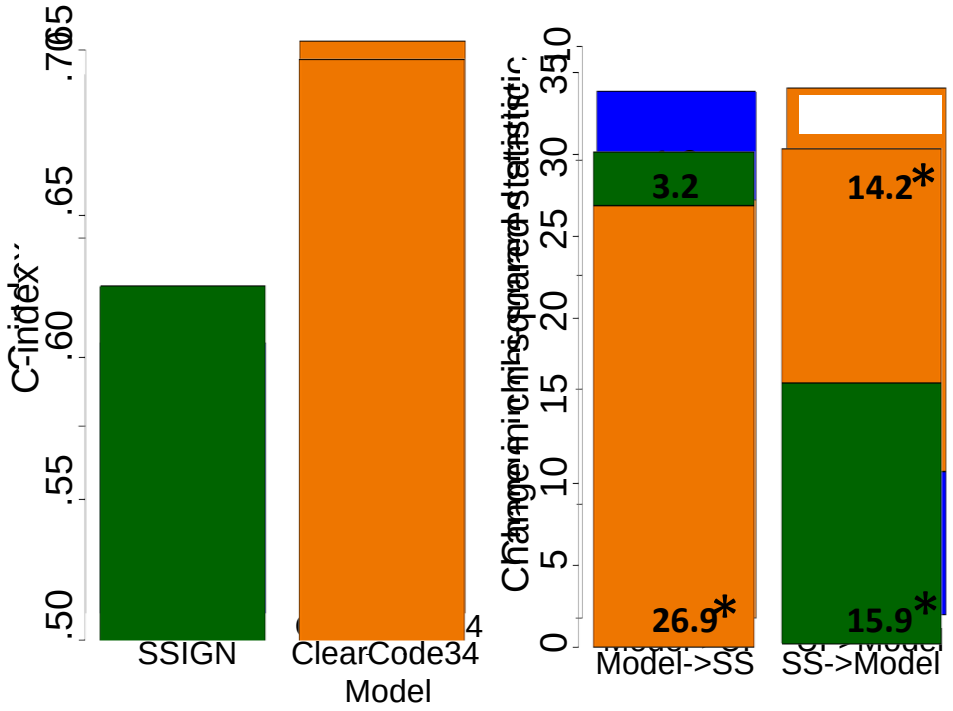
Integrated prognostic models can evaluate risk outcomes

Group	Risk Score
Low	0-0.5
Intermediate	0.5-1.5
High	>1.5





ClearCode34 Model outperforms established algorithms

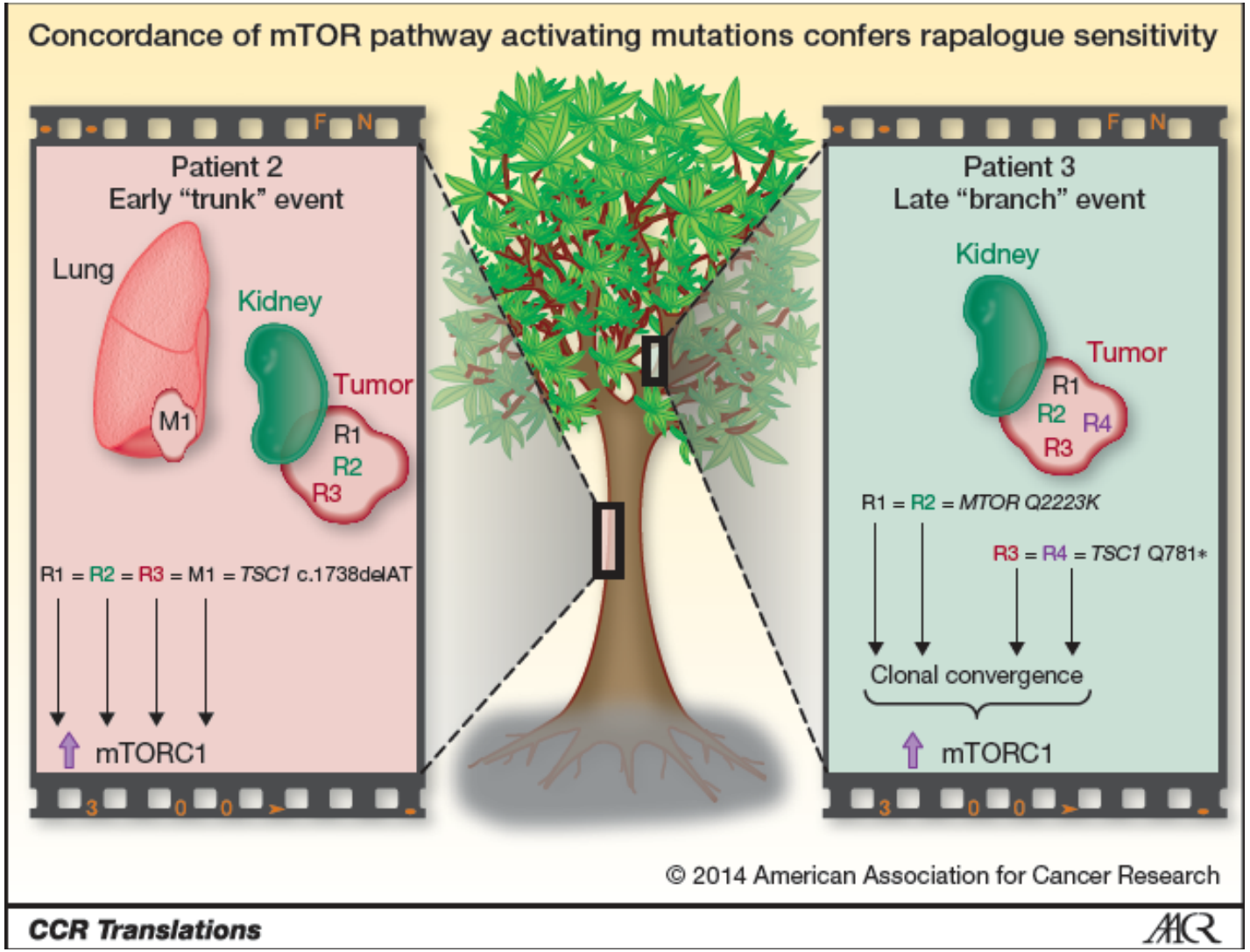


Summary

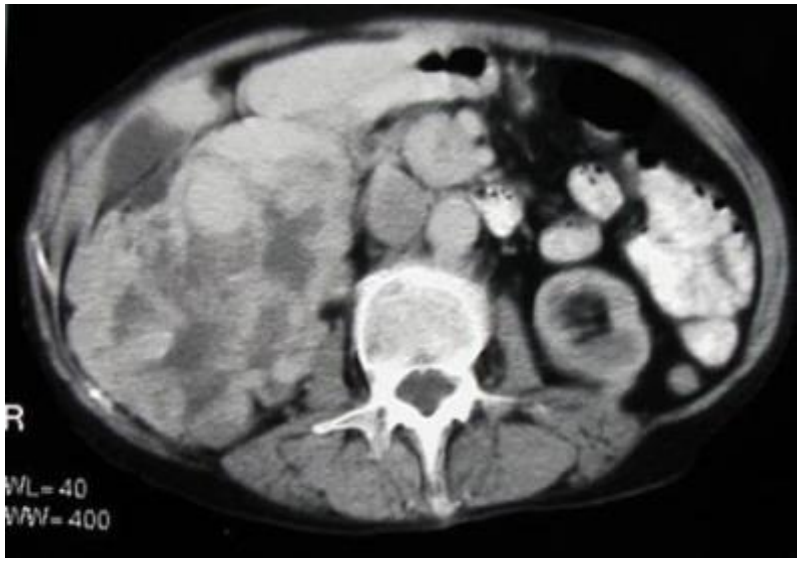
- Clear cell RCC can be divided based on gene expression into two groups.
- ccA and ccB tumors can be discriminated with 34 genes.
- A nanostring probeset allows assignment in fixed clinical specimens.
- Biomarkers add to clinical data in predicting risk assignments.

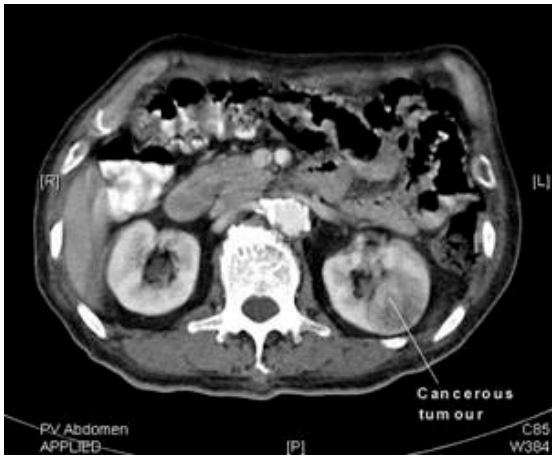
Heterogeneity-Hype, Hysteria, or Headache

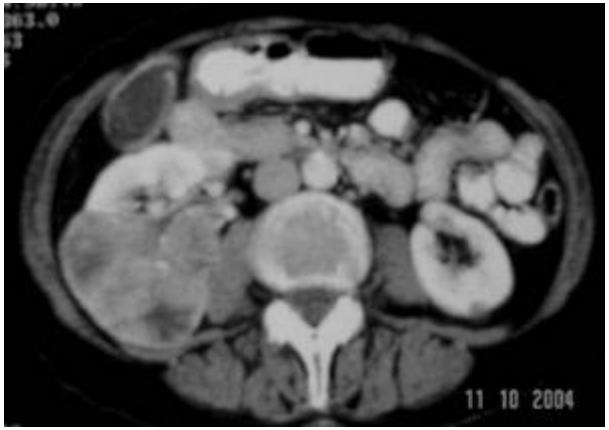
RCC tumors-heterogeneity, with convergent evolution

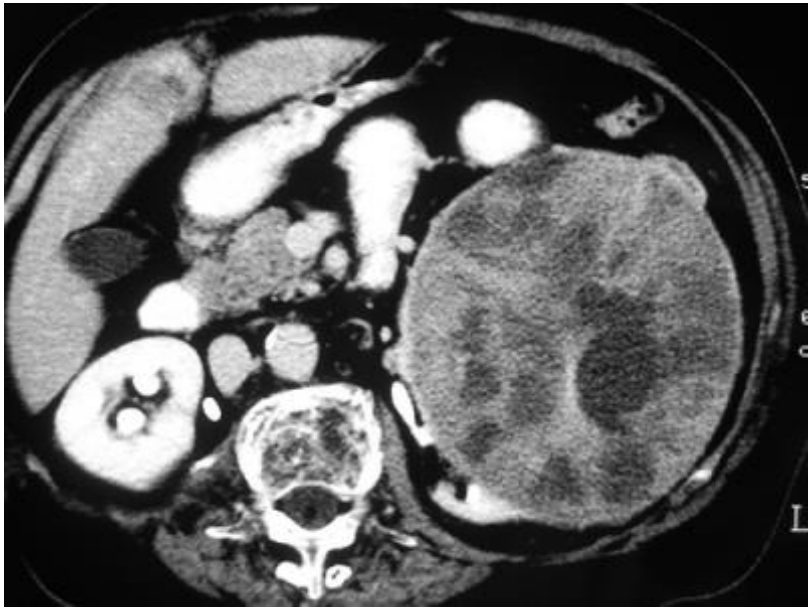


Images of renal tumors:



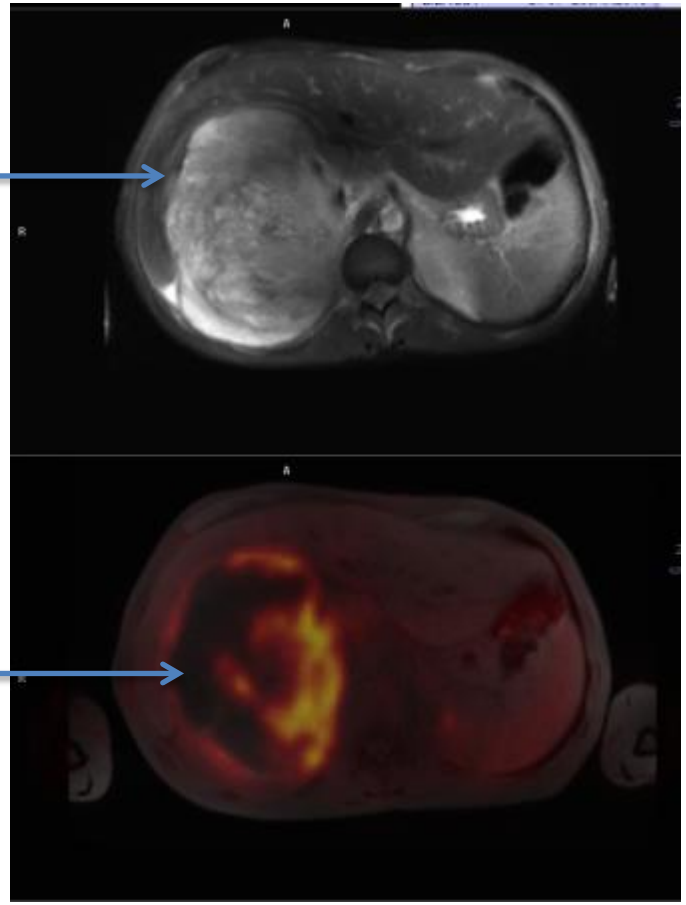






Imaging, another look at heterogeneity

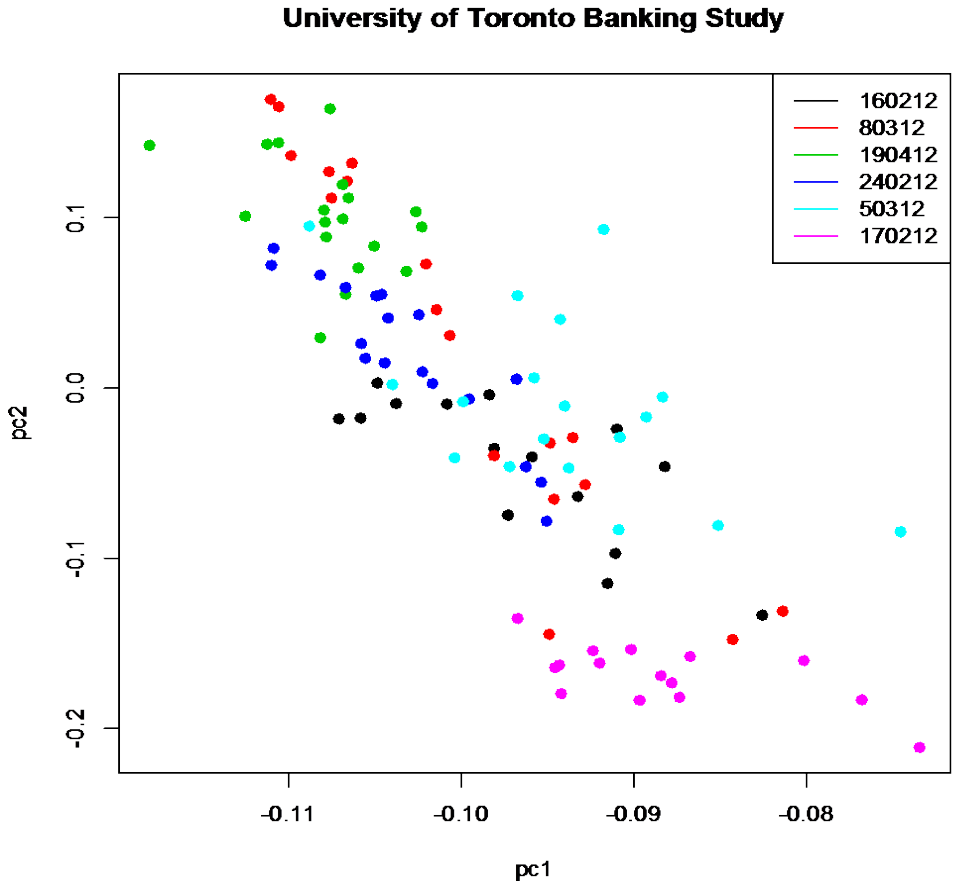
The same tumor can appear homogeneous by one method, and heterogeneous by another



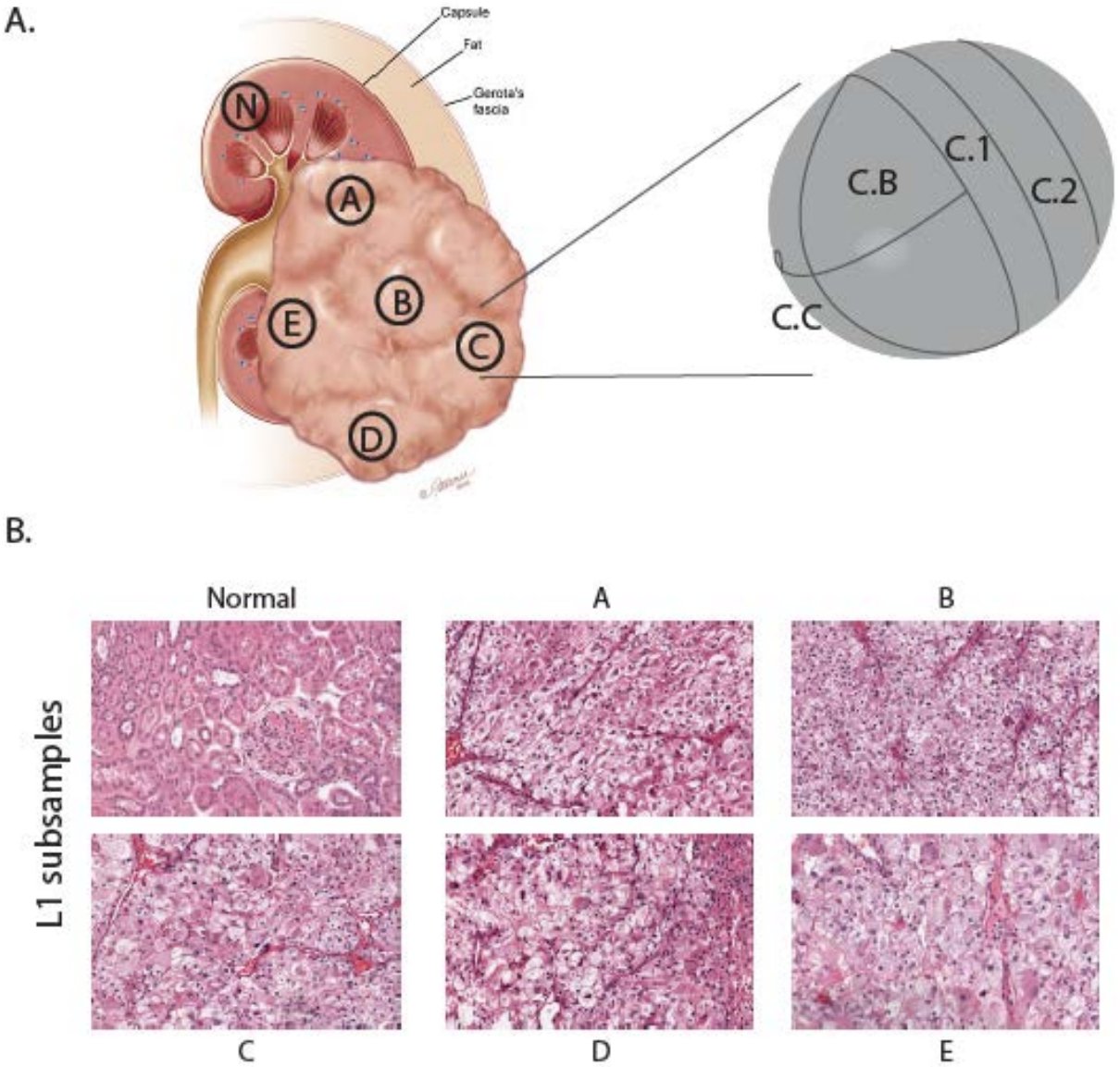
Measuring classes: Small renal masses

Low degree of gene expression heterogeneity in small tumors

Sample	Biopsies	No. Sublocations	Classification
160212	3	12	ccA
170212	3	12	ccA
240212	2	15	ccA
50312	3	15	ccA
80312	3	15	ccA
190412	3	15	ccA



A tale of one tumor.

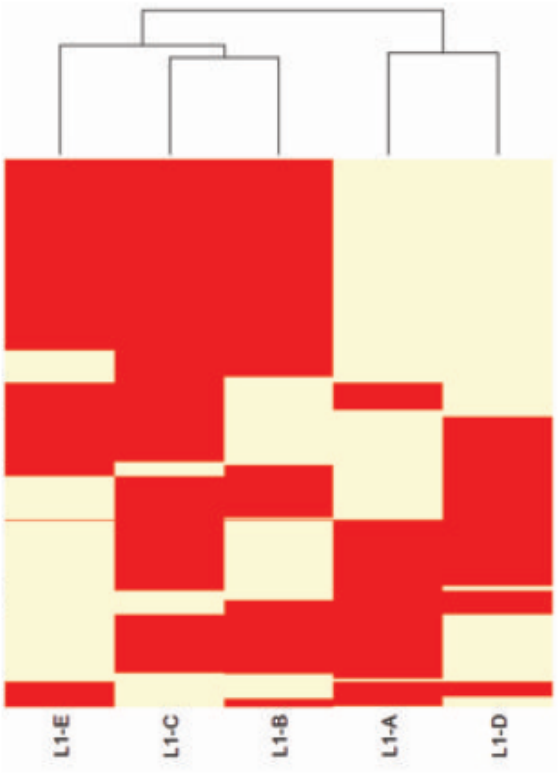


DNA Heterogeneity

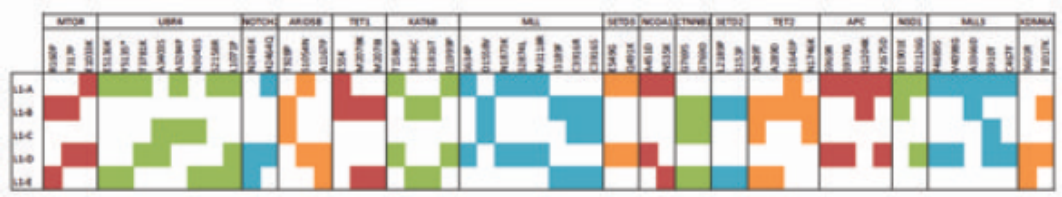
A.

Tumor	% reads mapped	% perfect pair
L1-A	93.4	80.5
L1-B	91.1	66.8
L1-C	88.4	49.5
L1-D	90.5	65.1
L1-E	89.2	56.7

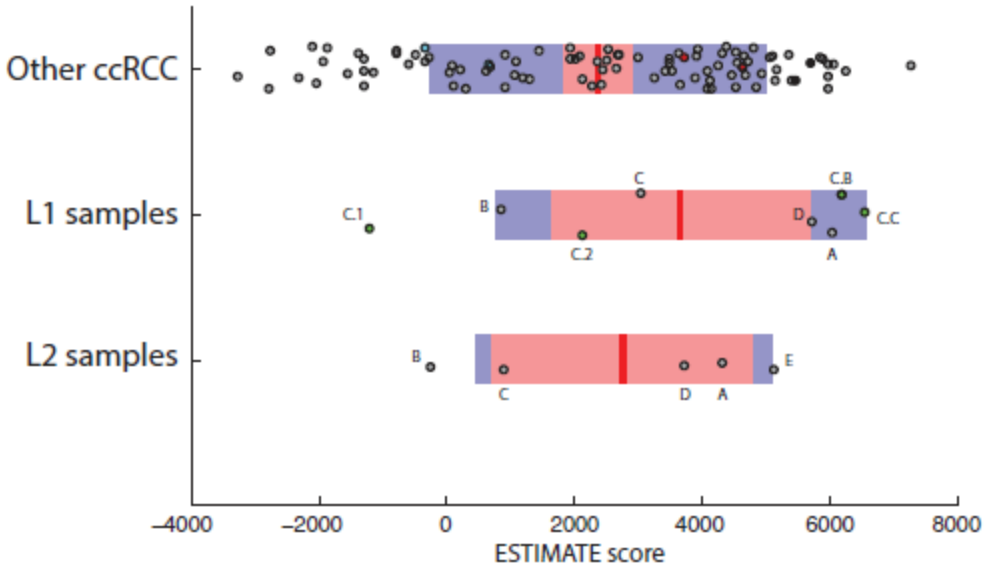
B.



C.

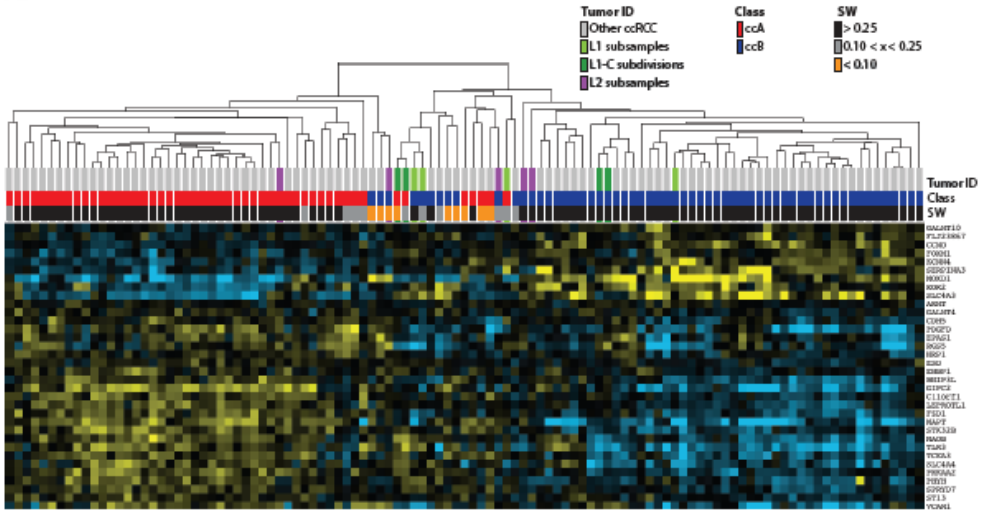


Effects of tumor purity.

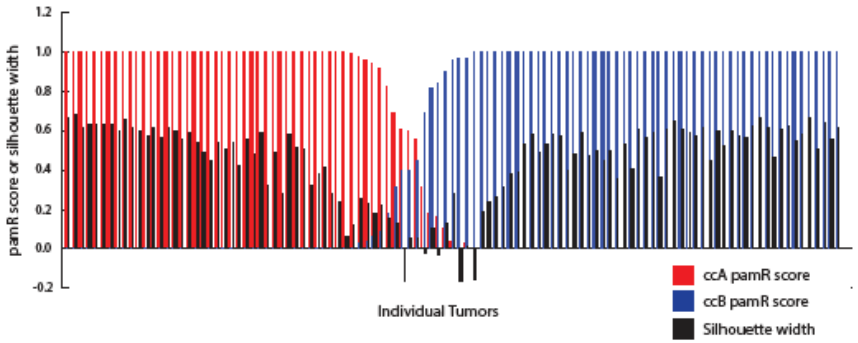


Heterogeneity of gene expression

A.



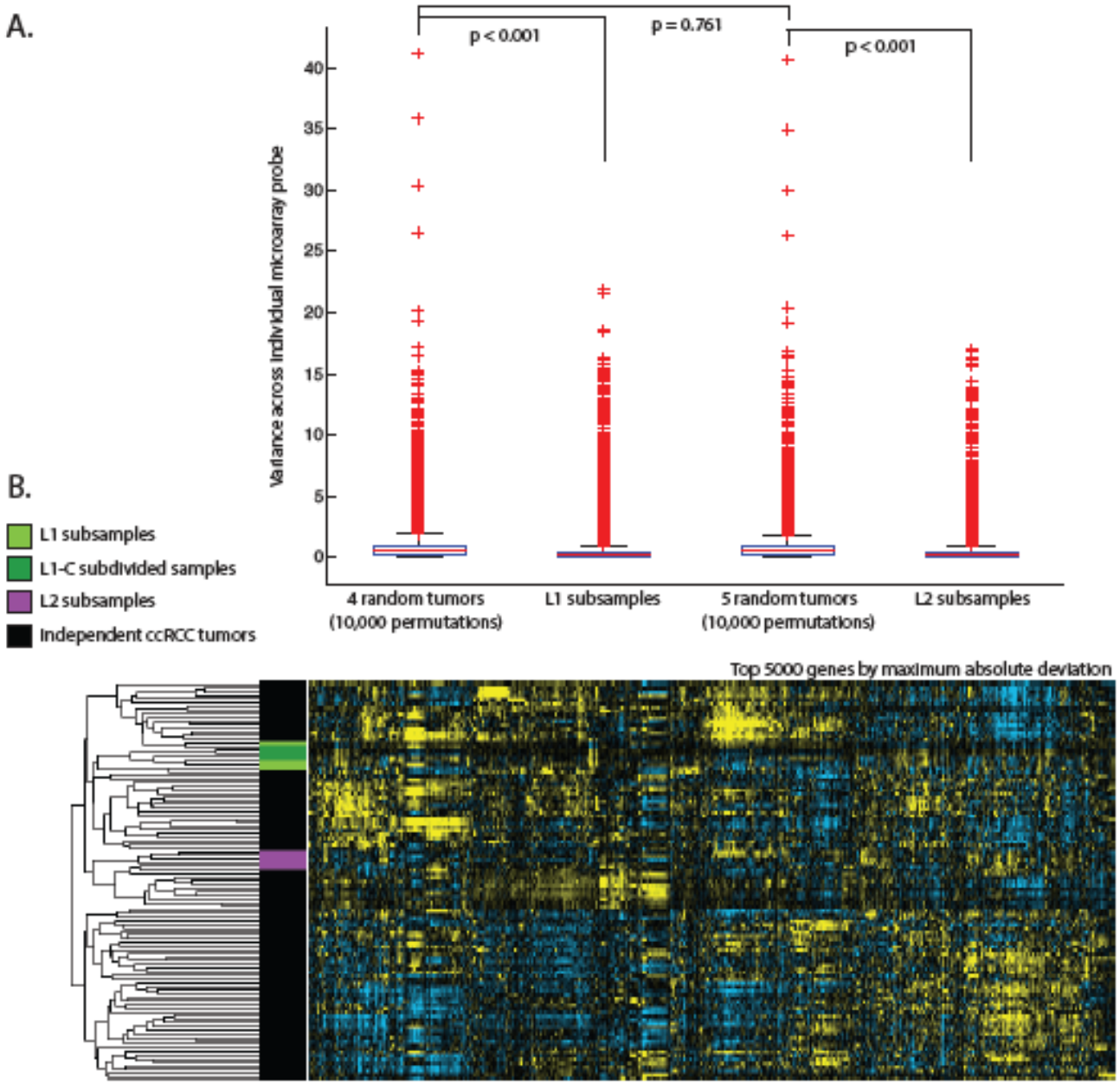
B.



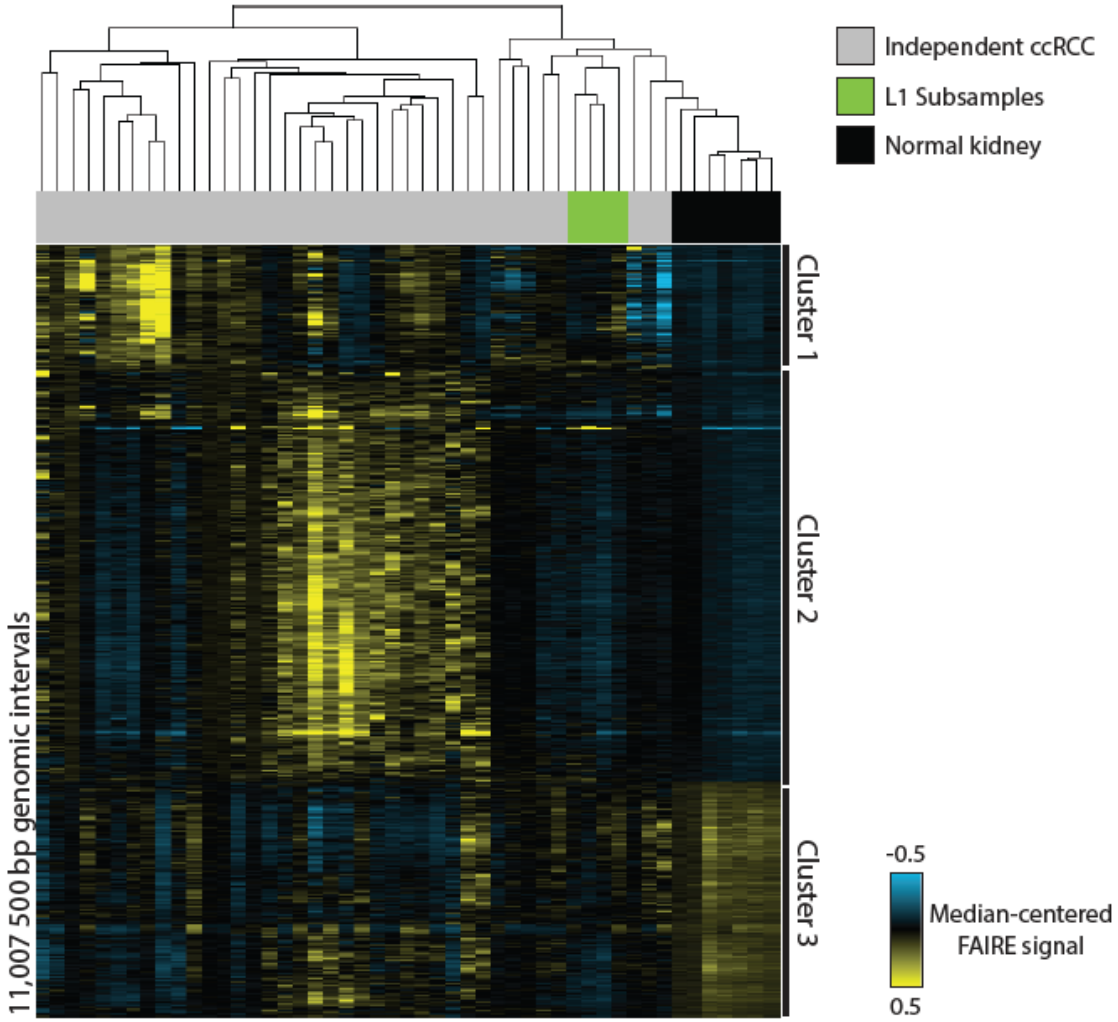
C.

Subsample ID	ccA PamR	ccB PamR	Class	Silhouette Width
L1.A	0.000	1.000	ccB	0.606
L1.B	0.001	0.999	ccB	0.237
L1.C	0.000	1.000	ccB	0.261
L1C.1	0.940	0.060	ccA	0.177
L1C.2	0.598	0.402	ccA	0.057
L1C.B	0.000	1.000	ccB	0.393
L1C.C	0.000	1.000	ccB	0.381
L1.D	0.685	0.315	ccA	0.129
L2.A	1.000	0.000	ccA	0.504
L2.B	0.004	0.996	ccB	0.192

Variance by gene expression



And the winner for most stable platform is…



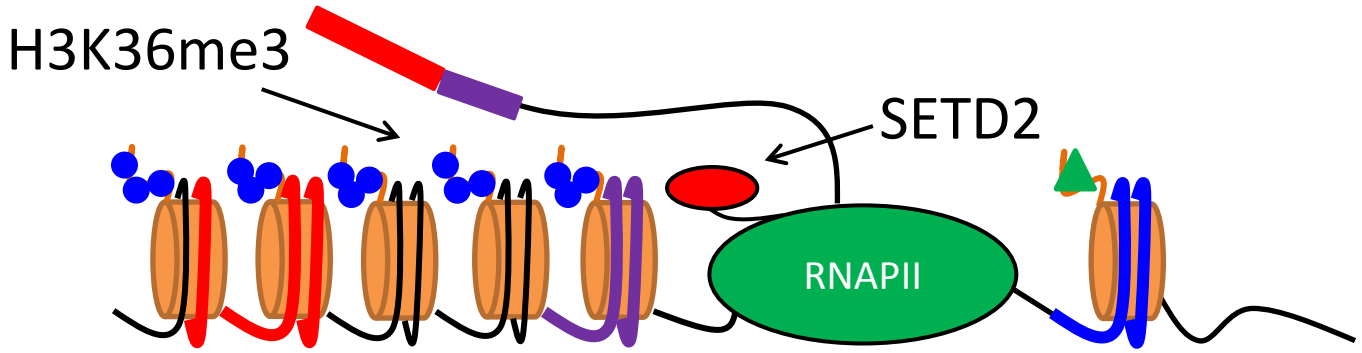
Summary

- Heterogeneity is the new normal, with pathway homogeneity (convergent mutations).
- Renal cell carcinomas can be heterogeneous, but this likely emerges with stage.
- Heterogeneity: DNA>RNA Biomarkers>RNA>enhancers
- Can imaging enable us to take a holistic view?

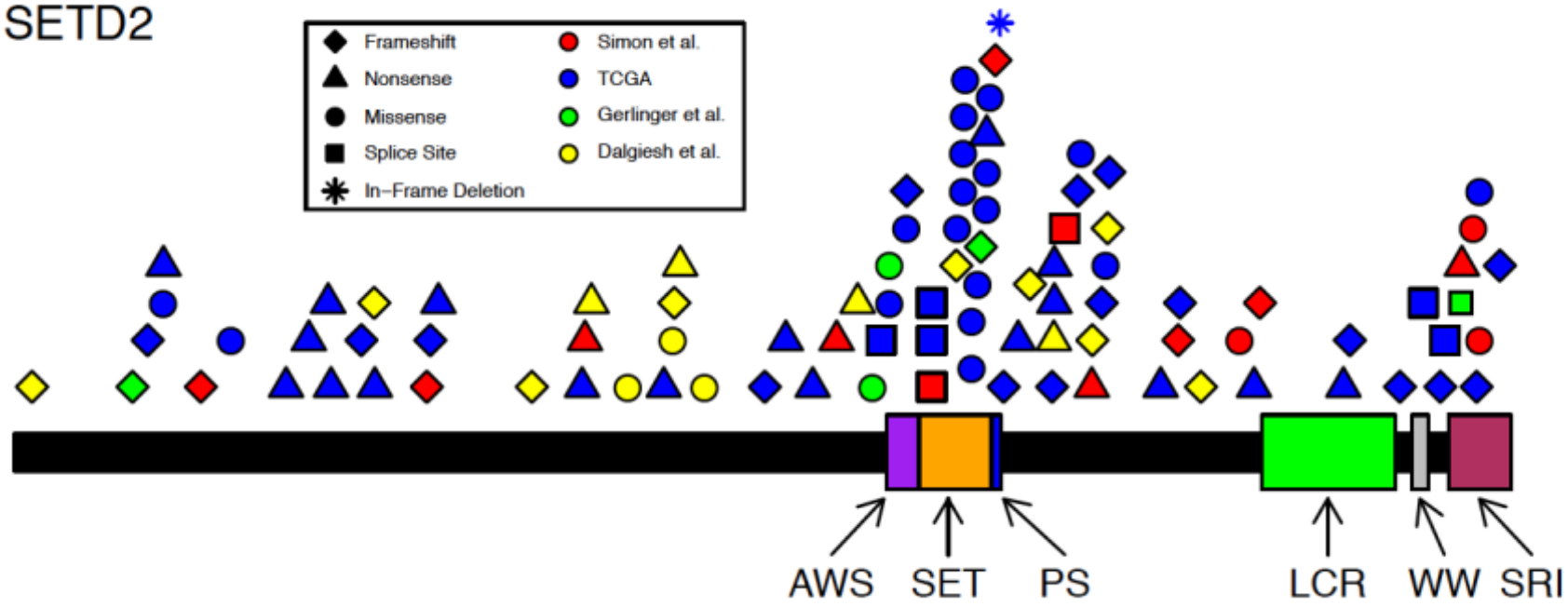
SETD2, transcription, and the histone code

"Unraveling" the cancer genome

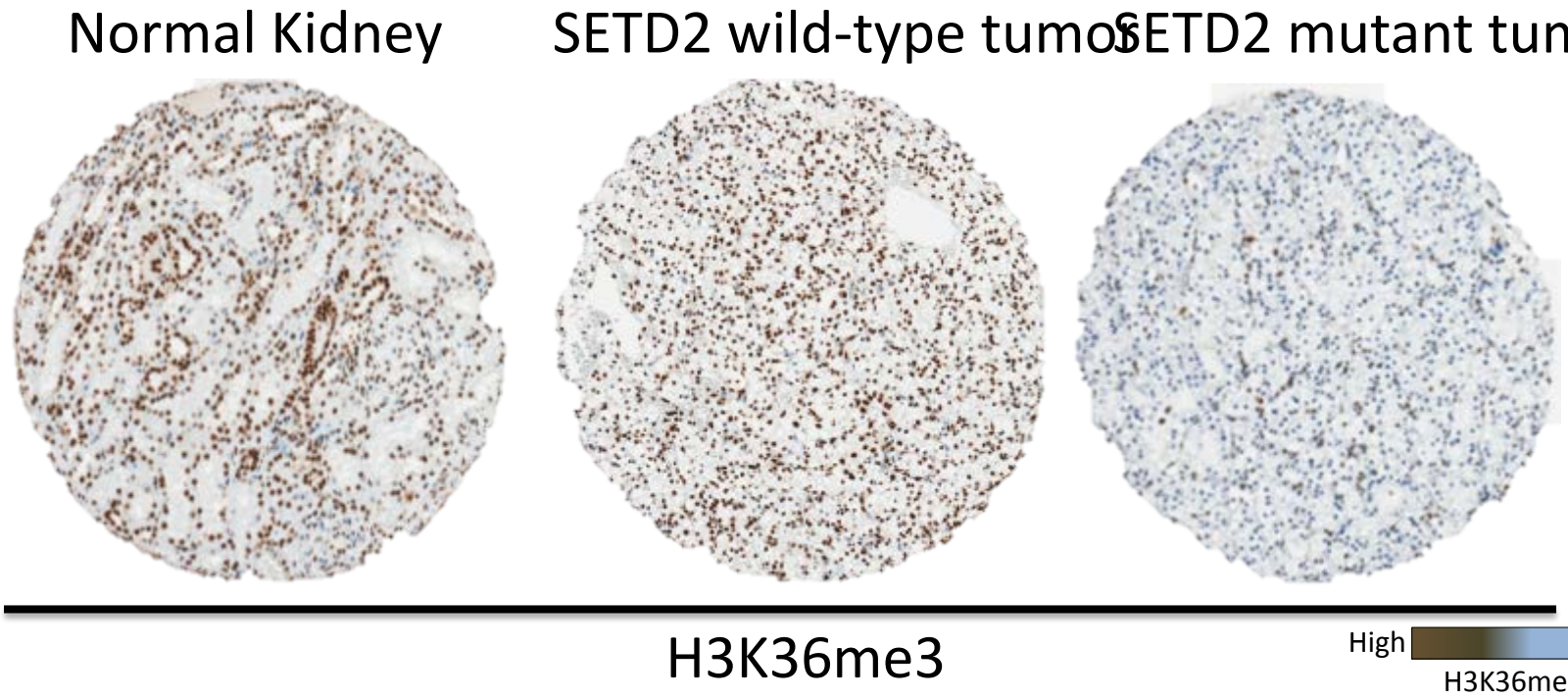
SETD2: a required H3K36 methyltransferase



SETD2 is mutated in ccRCC



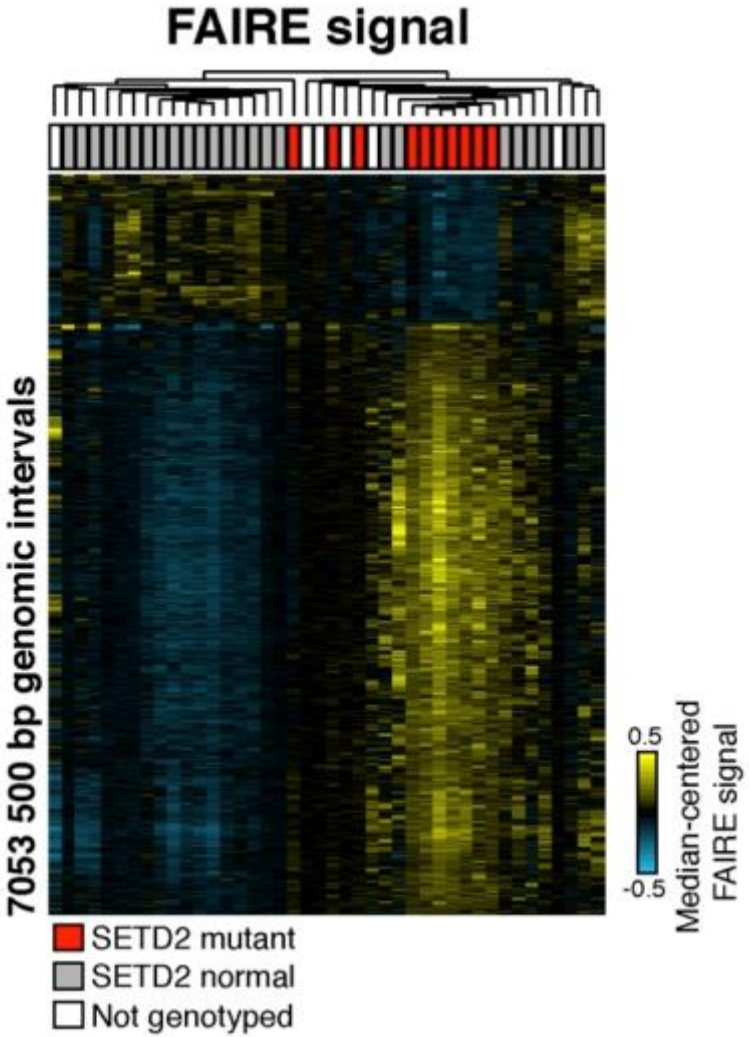
SETD2: a required H3K36 methyltransferase

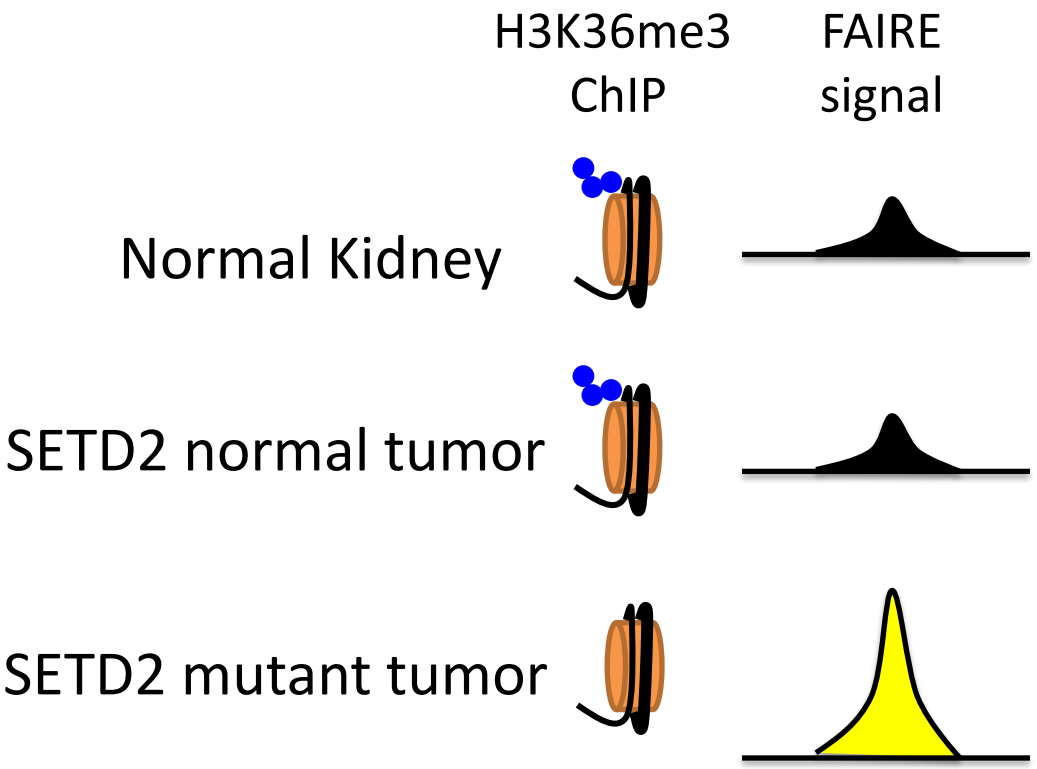


Loss of SETD2 has been shown to be associated with:

- Decreased global H3K36me3 levels
- Differential exon inclusion for individual genes (Luco et al., *Science* 2010)

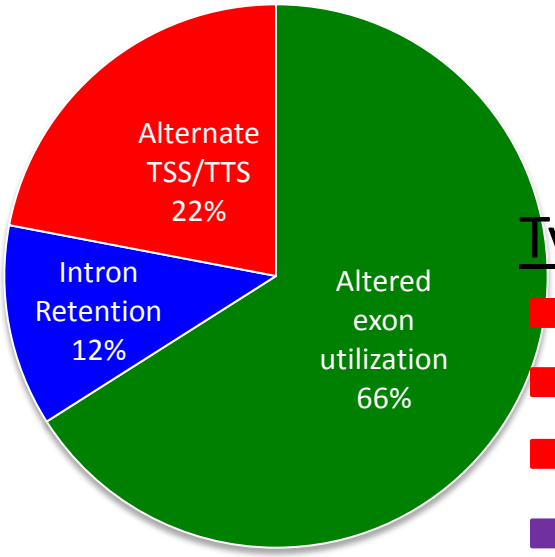
Tumors with SETD2 mutations display altered chromatin organization



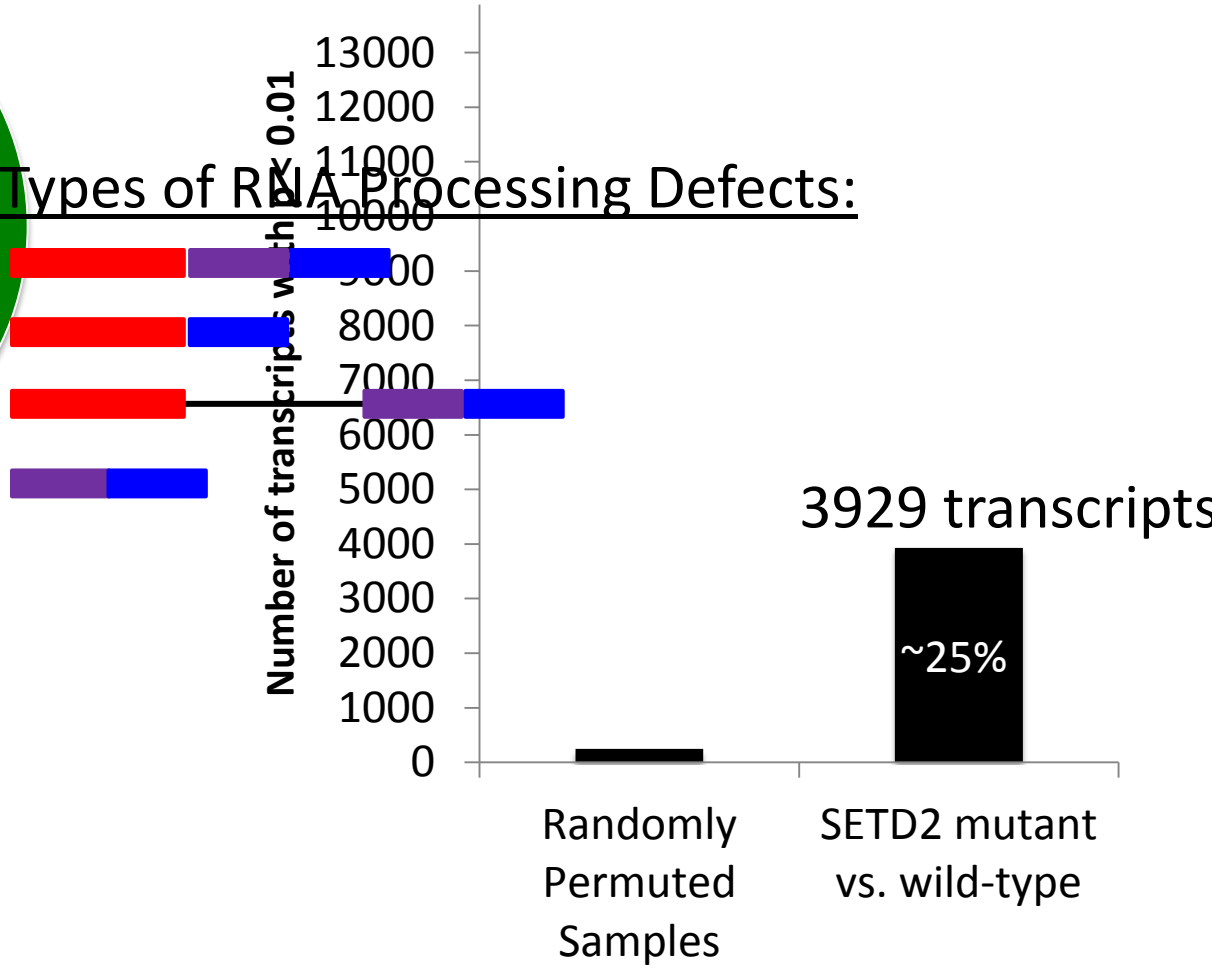


With Jeremy Simon

Tumors with SETD2 mutations display aberrant mRNA processing in poly(A)+ RNA

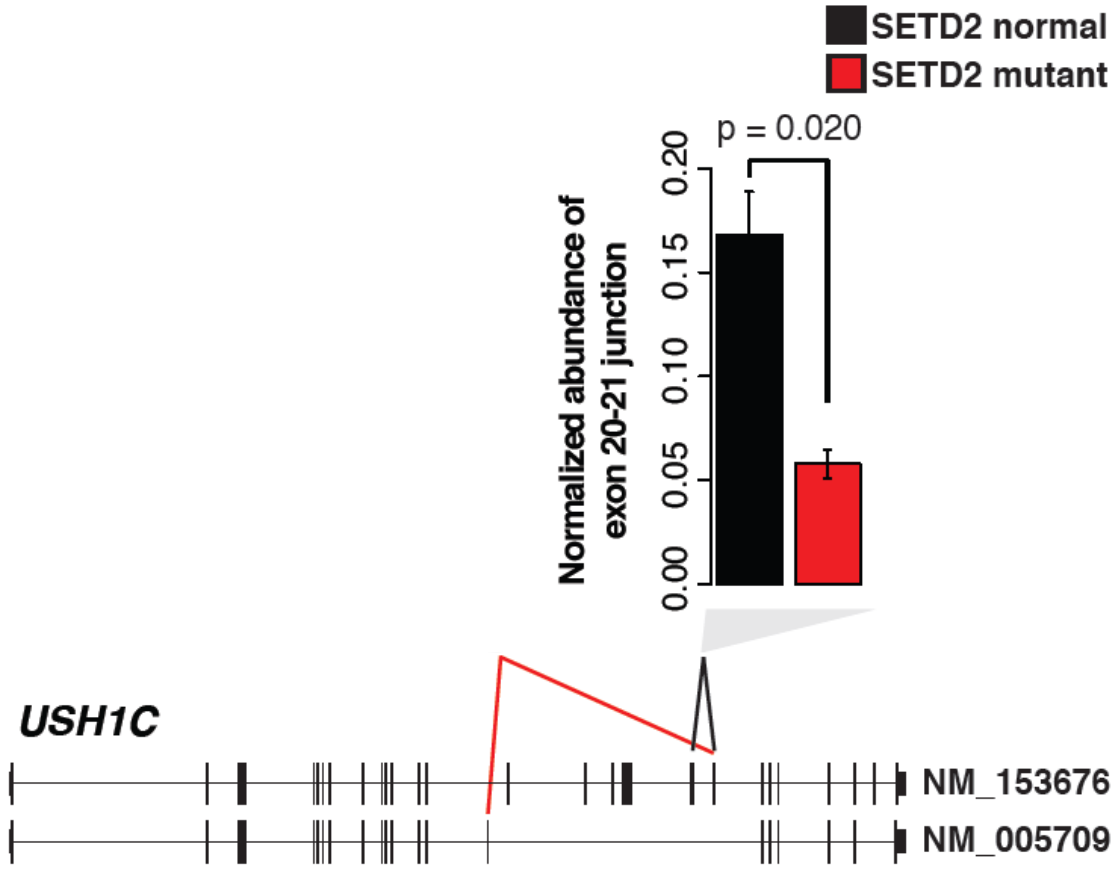


Types of RNA Processing Defects:

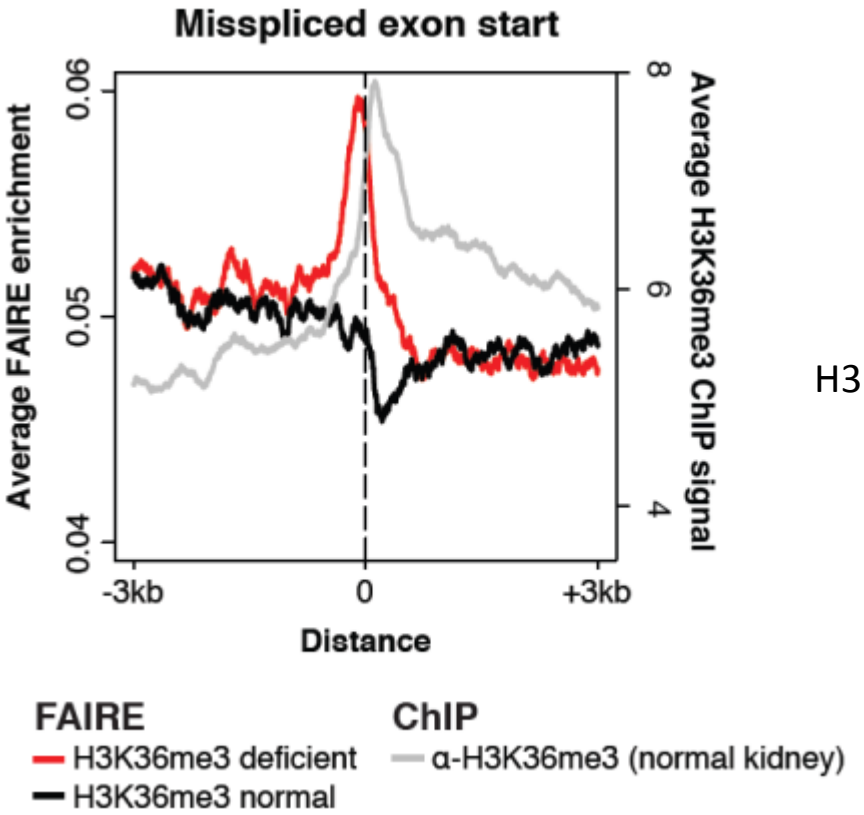


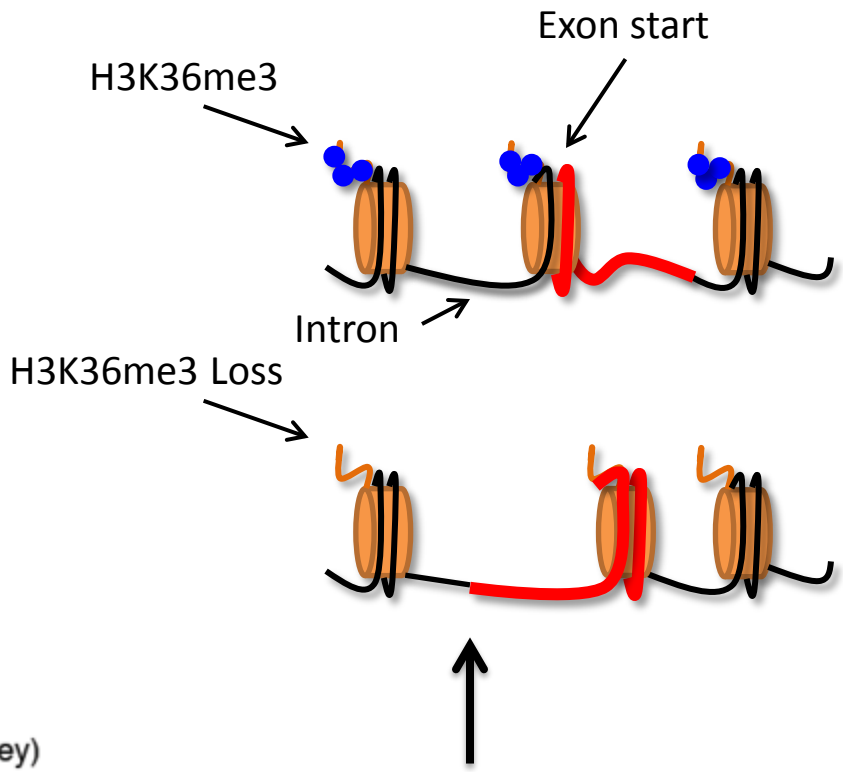
Darshan Singh, Jeremy Simon, Kate Hacket The Cancer Genome Atlas ccRCC

Tumors with SETD2 mutations display aberrant mRNA processing in poly(A)+ RNA

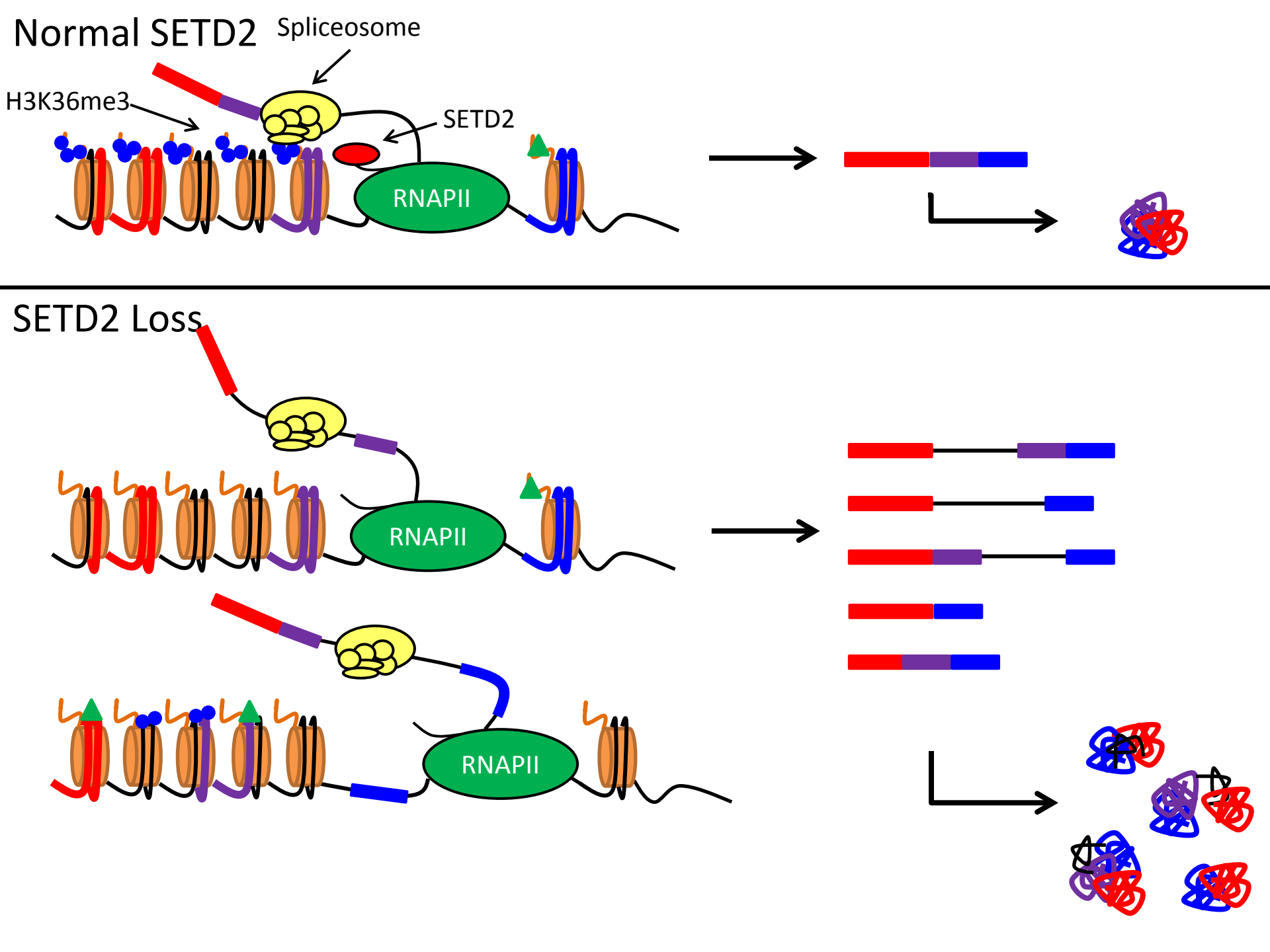


Sites of altered splicing display an increase in chromatin accessibility

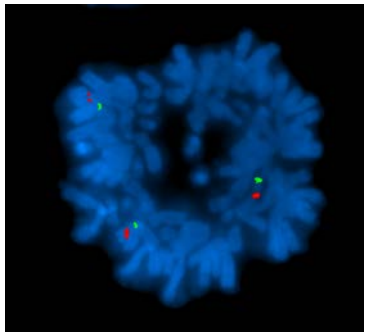




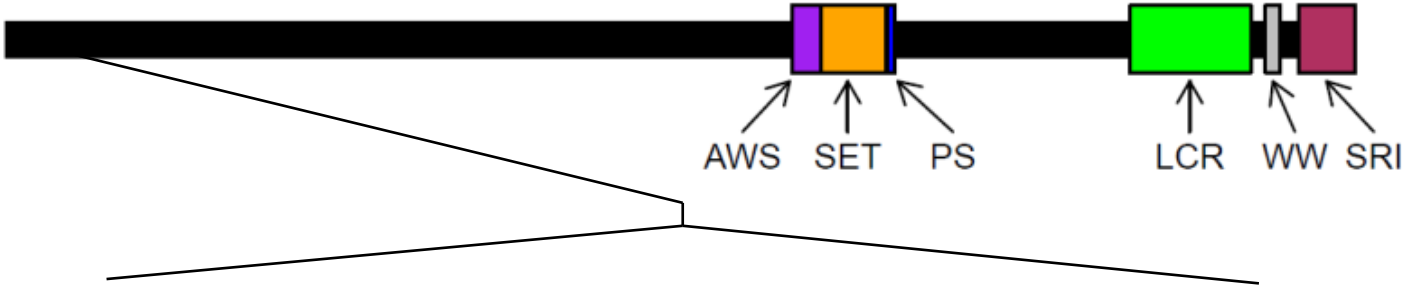
Open chromatin at misspliced


Normal SETD2
Spliceosome
H3K36me3
SETD2
RNAPII
SETD2 Loss
RNAPII
RNAPII

All three SETD2 alleles in 7860 cells are targeted



SETD2 Wild-type



Left Target | Spacer | Right Target

5′- **TCATGTAACATCCAGGCC**ACTGCTGGCTACTACC**ACAGCAGTAGCATCTCCA** - 3′

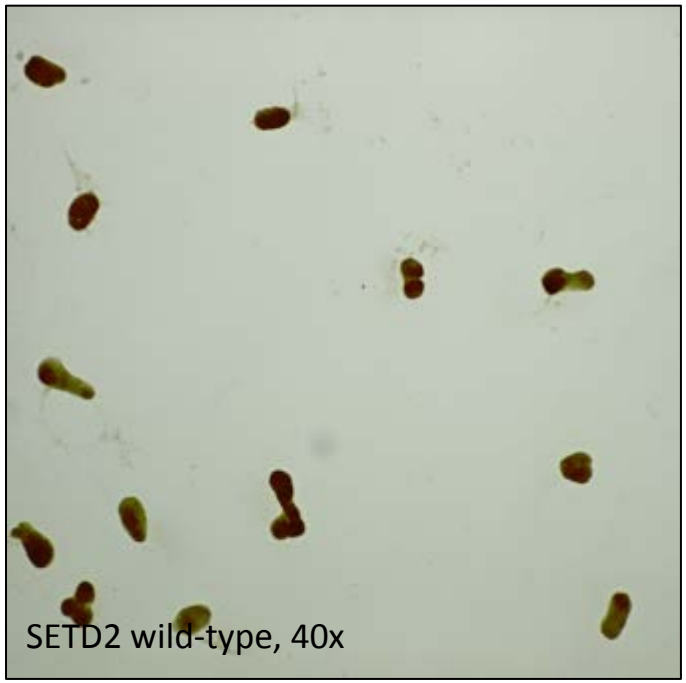
Allele: Representative SETD2 Inactivation

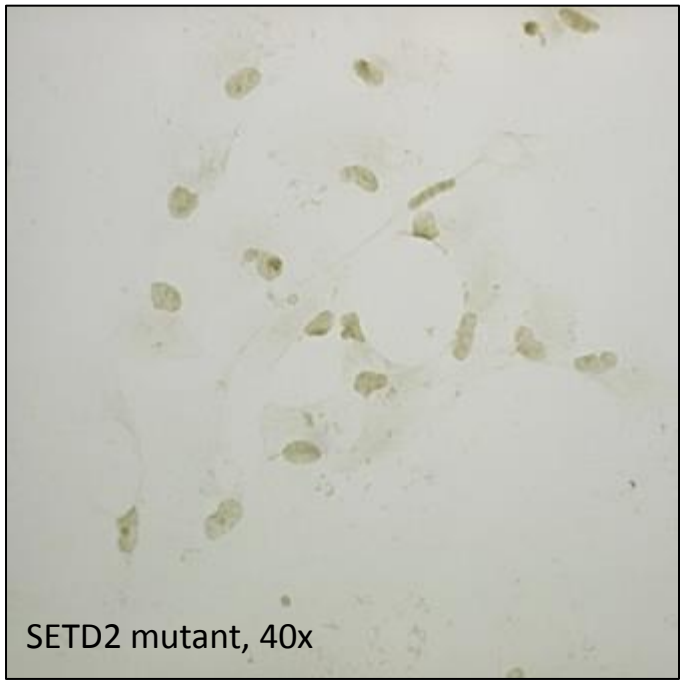
#1 5′- **TCATGTAACATCCAGGCC**ACTGCTGG----T----**CAGCAGTAGCATCTCCA** - 3′

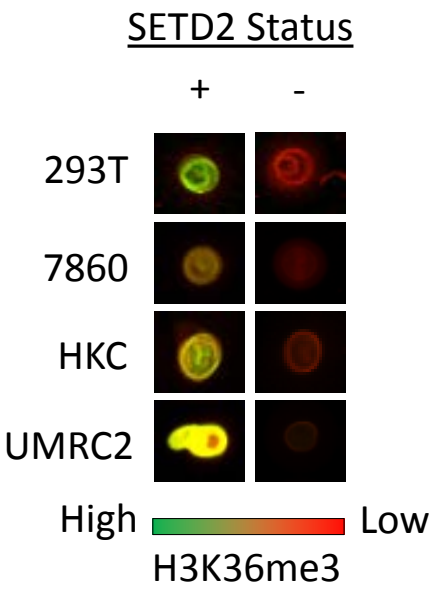
#2 5′- **TCATGTAACATCCAGGCC**ACTGCT----ACTACC**ACAGCAGTAGCATCTCCA** - 3′

#3 5′- **TCATGTAACATCCAGGCC**ACTGCTGGC-ACTACC**ACAGCAGTAGCATCTCCA** - 3′

Single cell sorting isolates SETD2 inactivated, H3K36me3-negative clones

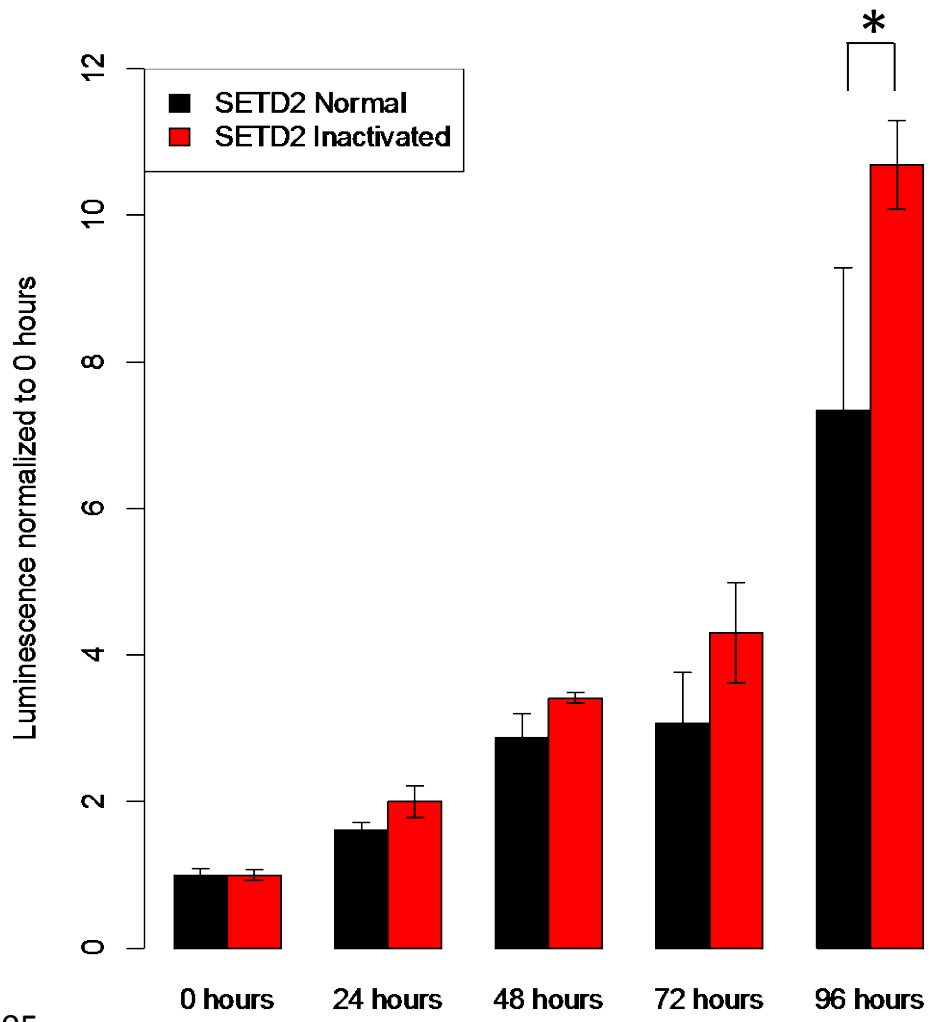






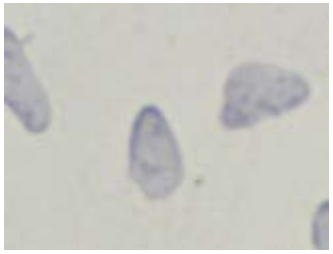
anti-H3K36me3

SETD2 loss increases cell proliferation



SETD2 WT

SETD2 KO



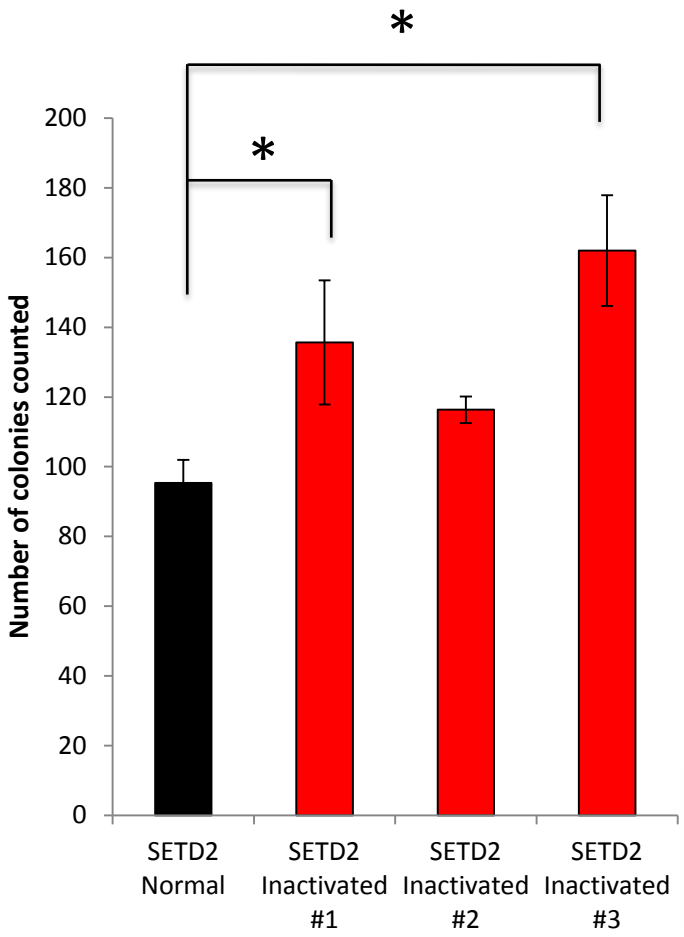
SETD2 KO+ tSETD2

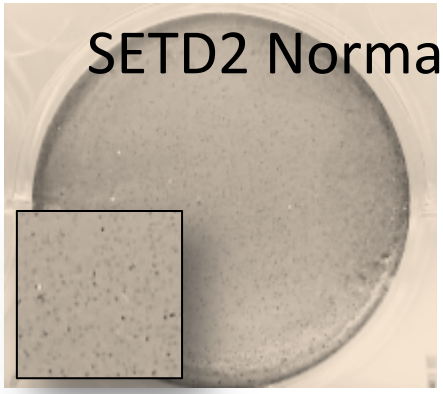
* $p < 0.05$

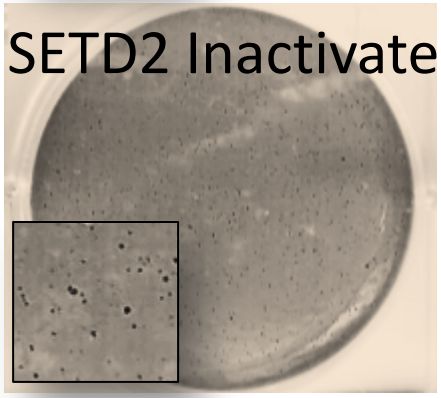
Shown = HKCs – human renal cell line with SV40 transformation

Confirmed in: 293Ts and 7860s (human ccRCC cell line)

SETD2 loss increases anchorage-independent growth



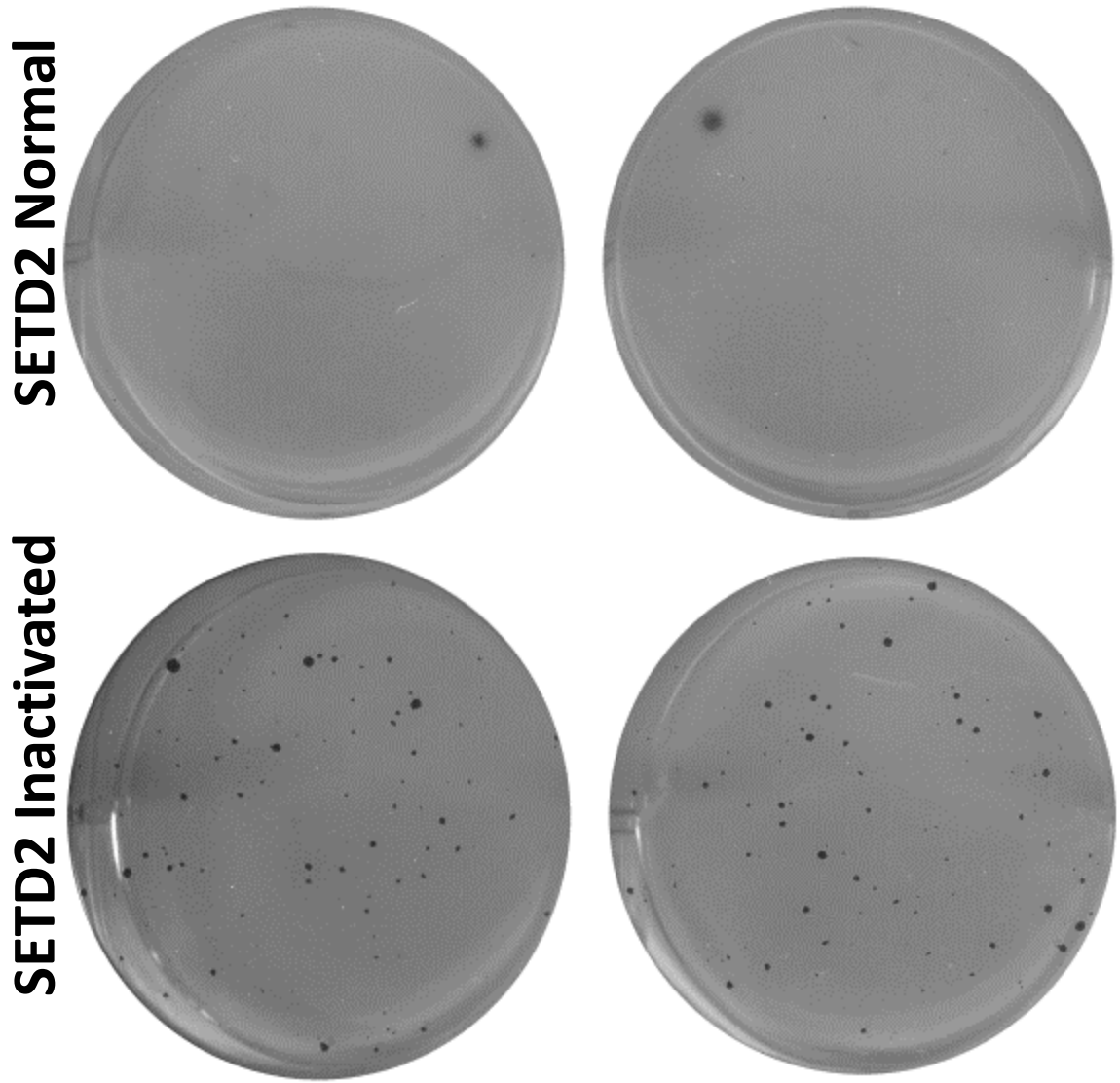


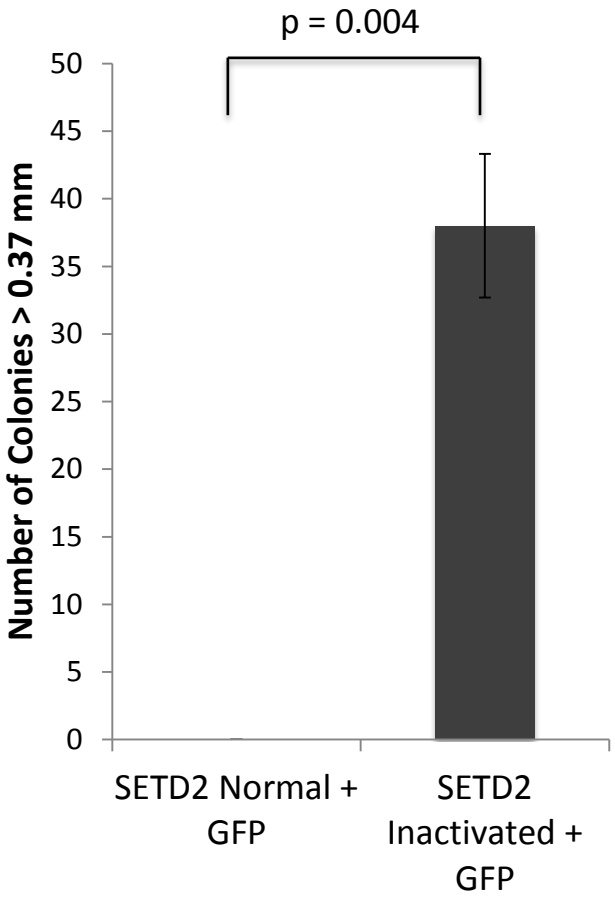


Cell line = 7860s

*p<0.05

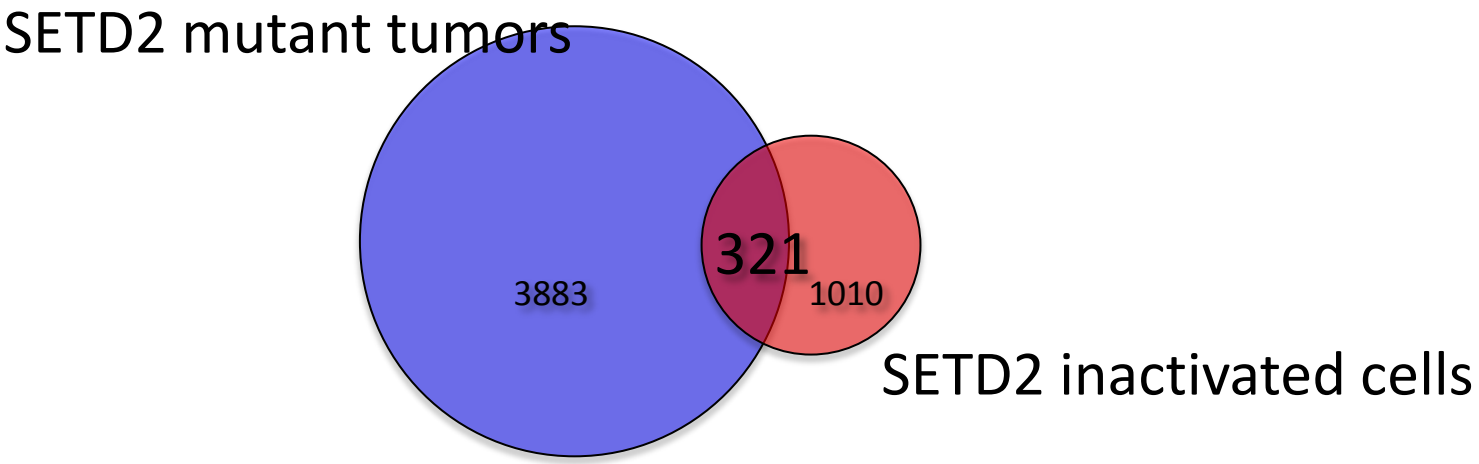
SETD2 loss is sufficient for anchorage-independent growth





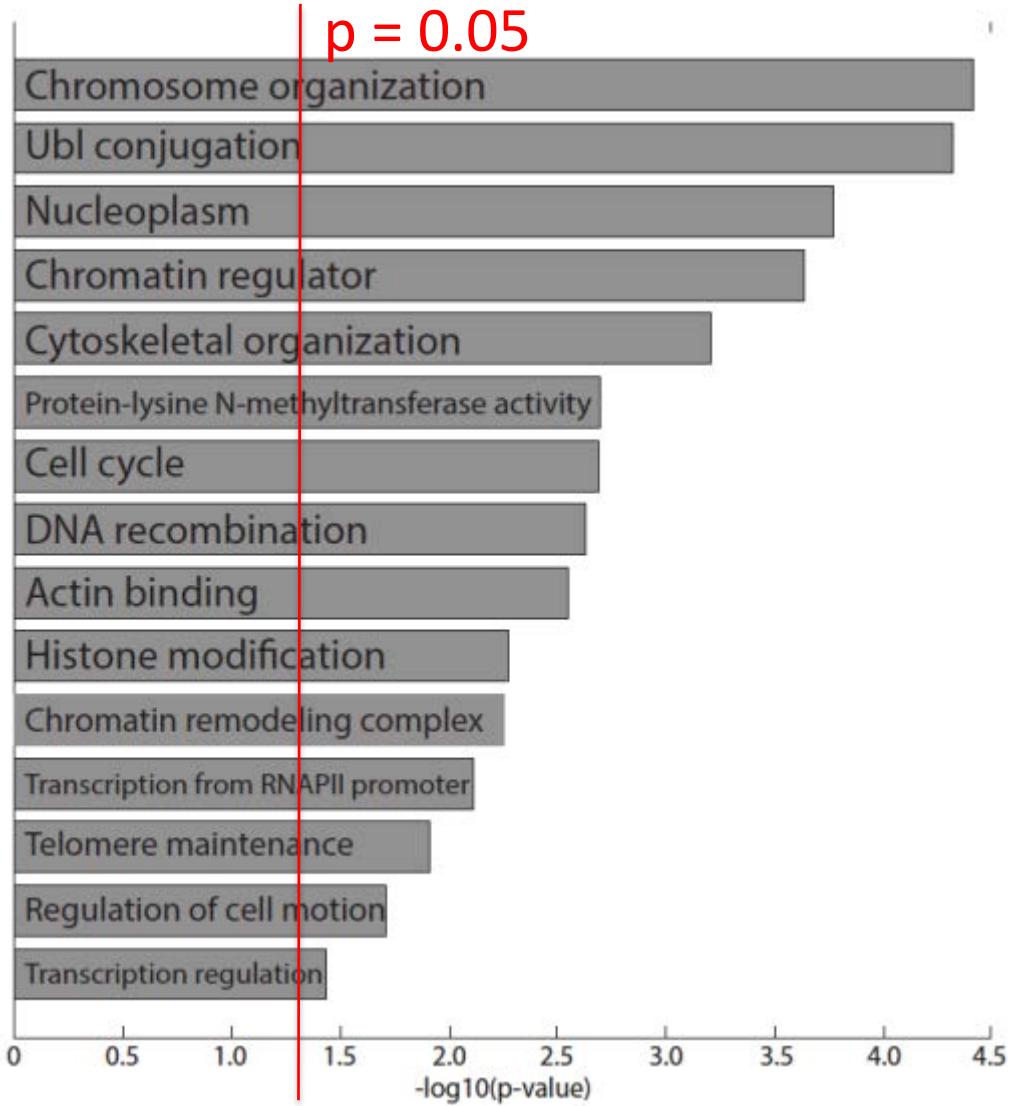
Cell line = HKCs

Isolated SETD2 loss results in widespread RNA processing defects



- 1269 aberrantly processed transcripts → 1010 individual genes
- ~32% overlap with aberrant transcripts in SETD2 mutant tumors
- Overlapping transcripts affect of wide variety of cellular processes

Isolated SETD2 loss results in widespread RNA processing defects



Summary

- SETD2 mutation is associated with changes in chromatin pattern and RNA processing.
- Association with loss of nucleosome at misspliced exon starts.
- SETD2 loss confers a proliferative and survival advantage.

Acknowledgments

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