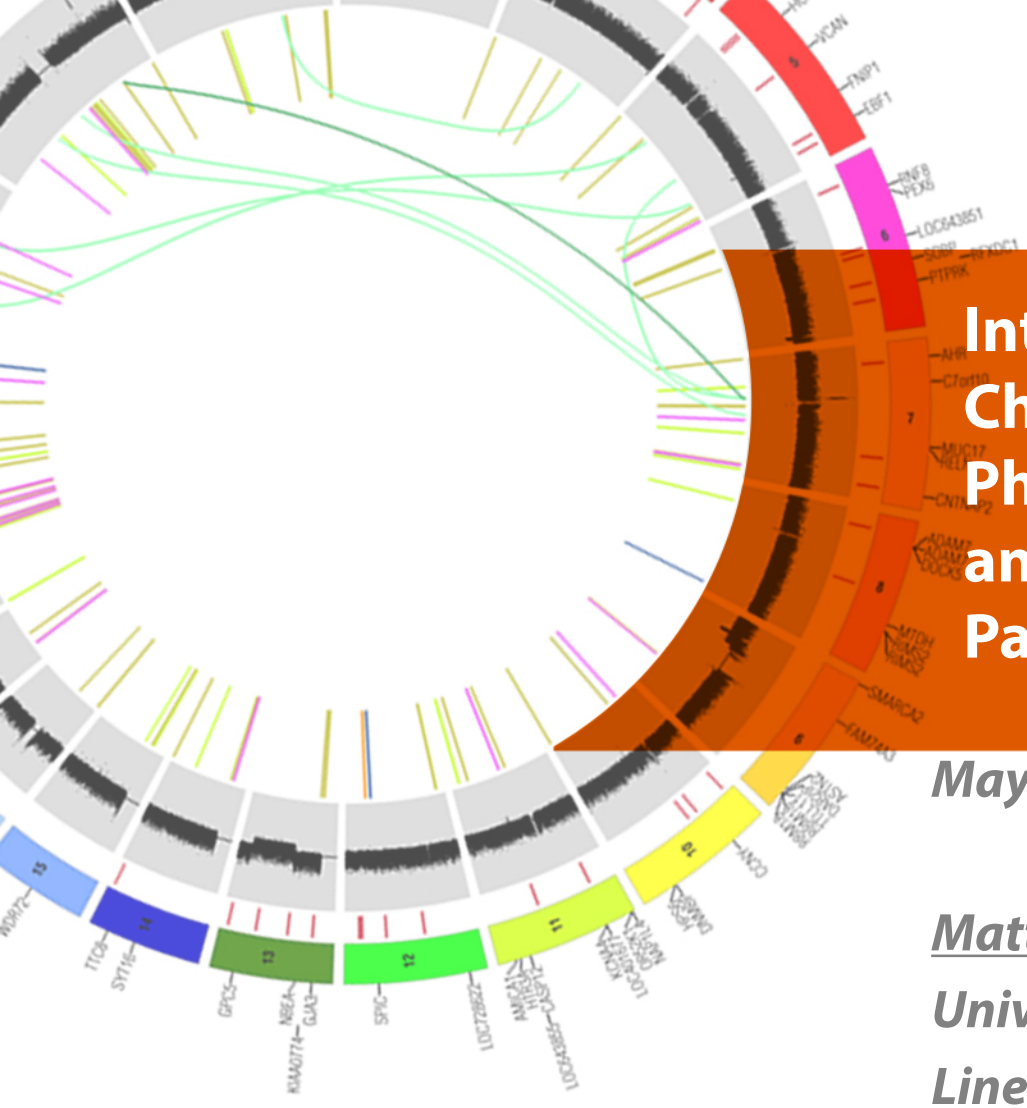

The Cancer Genome Atlas

# Integrated Genomic Characterization of Pheochromocytoma and Paraganglioma

*May 11, 2015*


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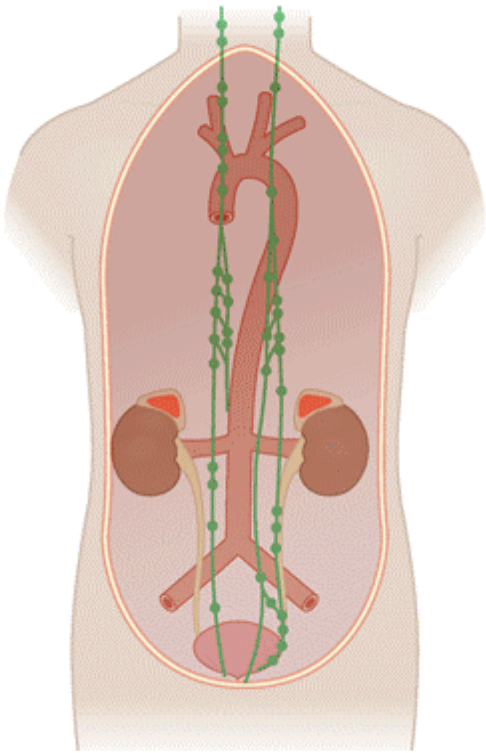
*Lineberger Comprehensive Cancer Center*

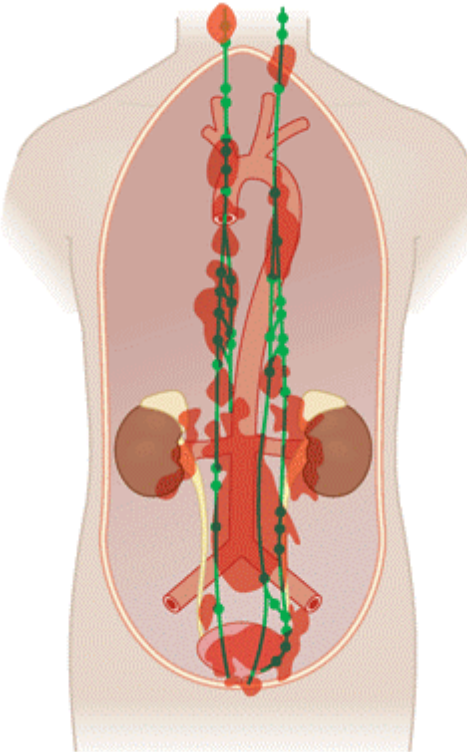
*On behalf of the Pheochromocytoma TCGA analysis working group*

# Background on Pheochromocytoma and Paraganglioma

- Rare chromaffin cell neoplasm
  - 1,000 cases per year in the U.S.
- Chromaffin cells: neuroendocrine cells secrete catecholamines (e.g. epinephrine)
  - Named by location
- For brevity, I'll use "Pheo" in speaking to refer to both Pheochromocytoma and Paraganglioma.
- Up to 30% malignant (Tischler et al, 2014, Endocrine Pathology)
  - For malignant disease, 46% progression-free survival at 1 year (Hescot, 2013)
  - Few markers of metastatic risk



Adrenal medulla:
**Pheochromocytoma**

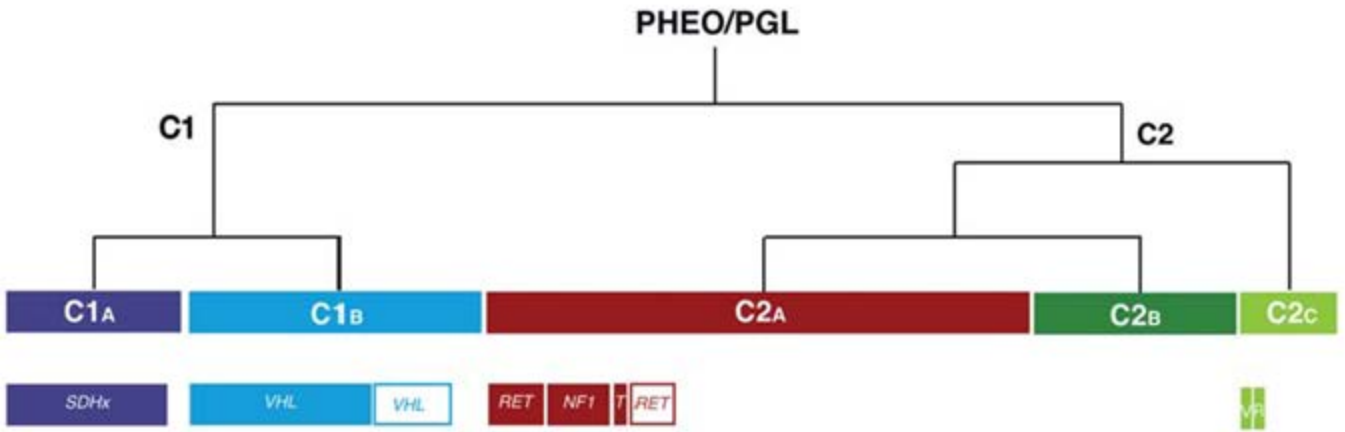


Extra-Adrenal:
**Paraganglioma**

Harrison's Principles of Internal Medicine

# Background on Pheo Genetics and Genomics

- Underlying inherited mutations in ~ 40% (highest of any tumor type)
  - (Dahia, 2014 Nature Genetics, Fishbein et al, 2013 Ann Surg Oncol)
- 19 susceptibility genes
  - *NF1, RET, SDHB, SDHD, SDHA, SDHC, MAX,* other less frequent genes.
- Pheo can be familial or sporadic.
- mRNA Expression Clusters
  - Pseudohypoxia and Kinase signaling (Dahia, 2005, Plos Genetics)
  - Potentially up to five clusters (Burnichon)
  - Associate with different susceptibility genes.



# The Pheo Cancer Genome Atlas

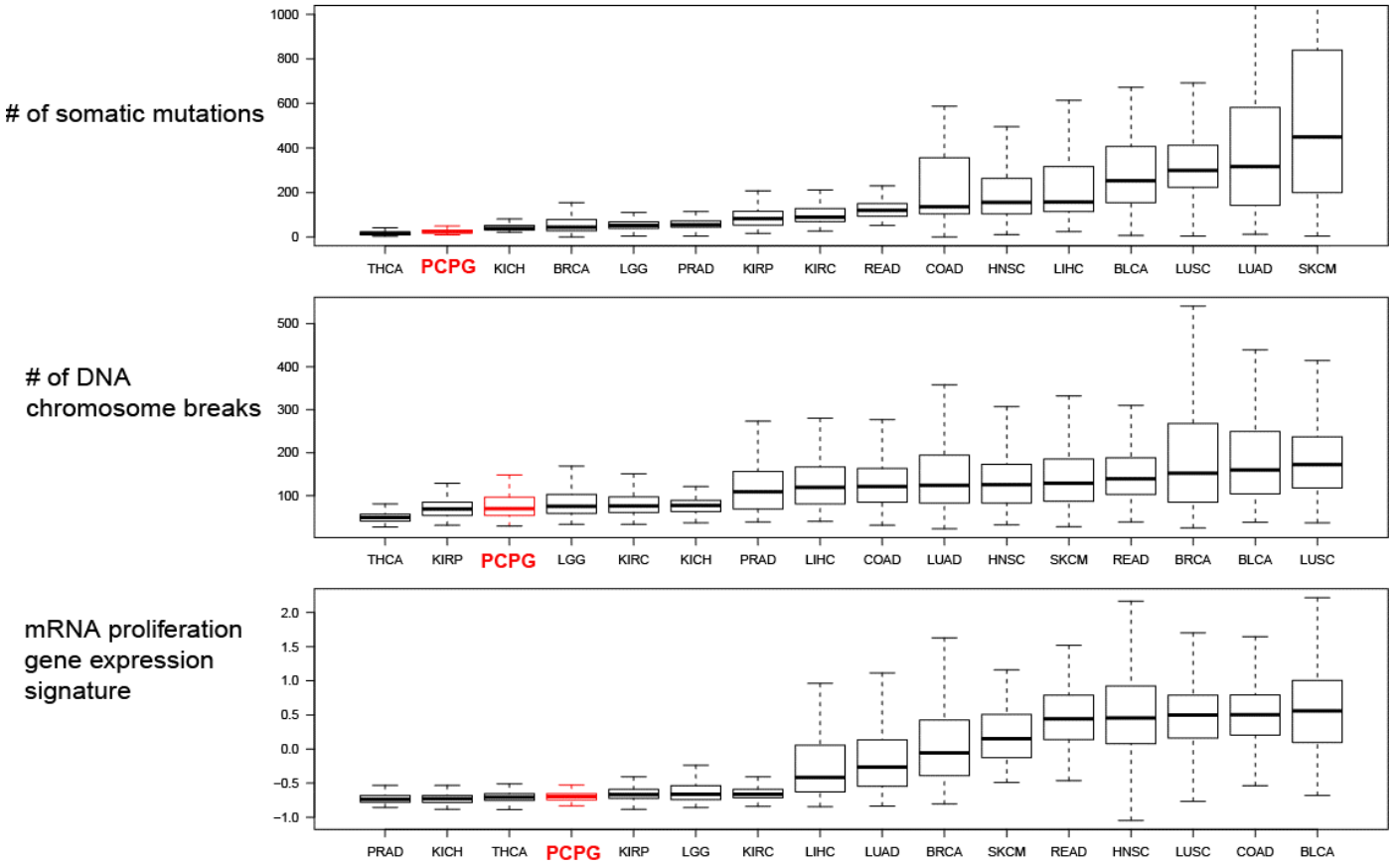
- Aim is to identify Pheo's
  - Genomic alterations
  - Integrated classifications
  - Markers of benign vs metastatic disease

- Cohort: 173 patients

- Each case has the following assays, on tumor (T) or germline (G) tissue

| | | |
|---|---|---|
| – DNA Whole Exome Sequencing | T | G |
| – DNA copy number arrays | T | G |
| – mRNA sequencing | T | |
| – miRNA sequencing | T | |
| – DNA methylation arrays | T | |
| – Reverse Phase Protein Arrays (cohort subset) | T | |

# On a large scale, Pheo has a quiet somatically altered genome




- Also among lowest by DNA methylation alterations (not shown)

# Pheo has diverse mutations

Ignat Leshchiner
Kate Nathanson
Brandon Wenz
Matt Wilkerson

Germline mutations

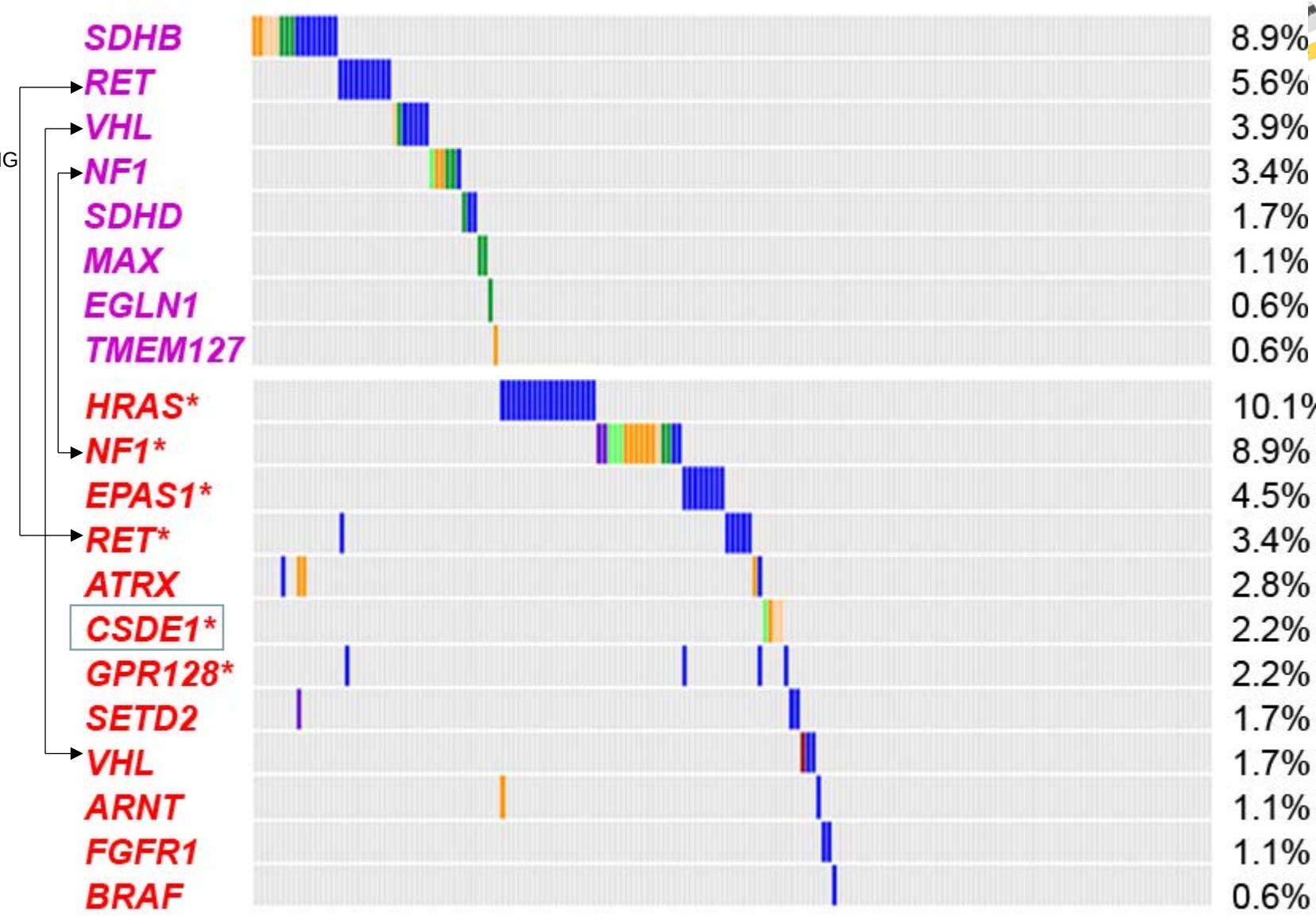
(classified as pathogenic / likely pathogenic by ACMG guidelines)

**> 25 % positive**

Somatic Mutations

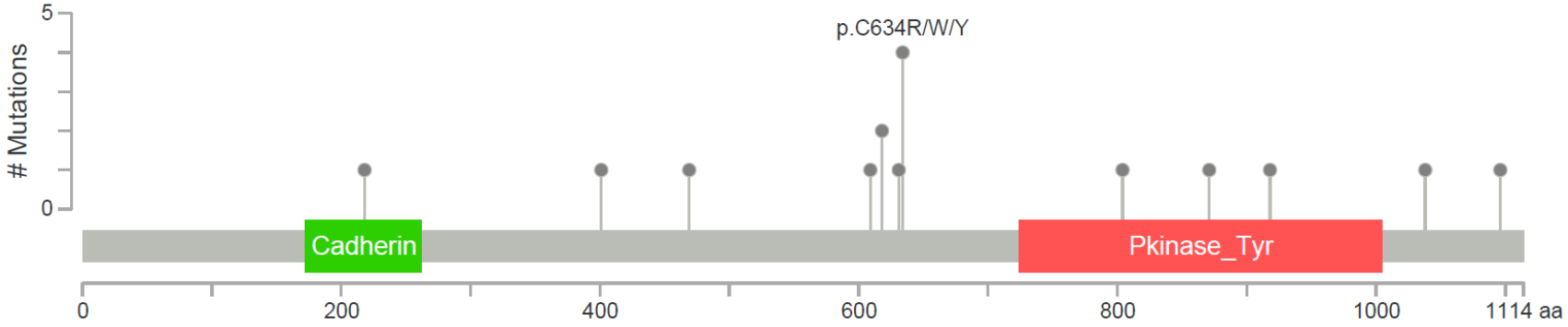
(* MutSig q<0.1)

**> 40% positive**

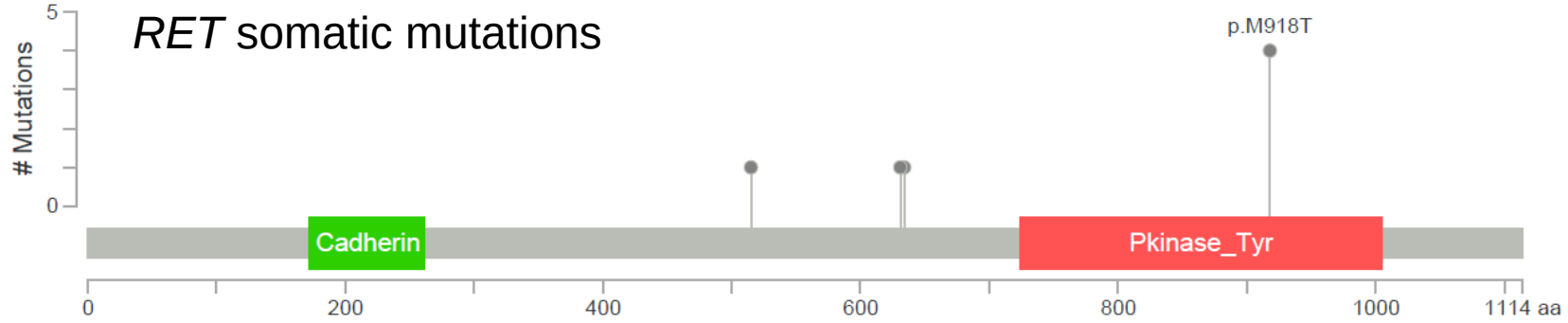


# *RET* mutation tendencies vary by germline or somatic mutation origin

*RET* germline mutations



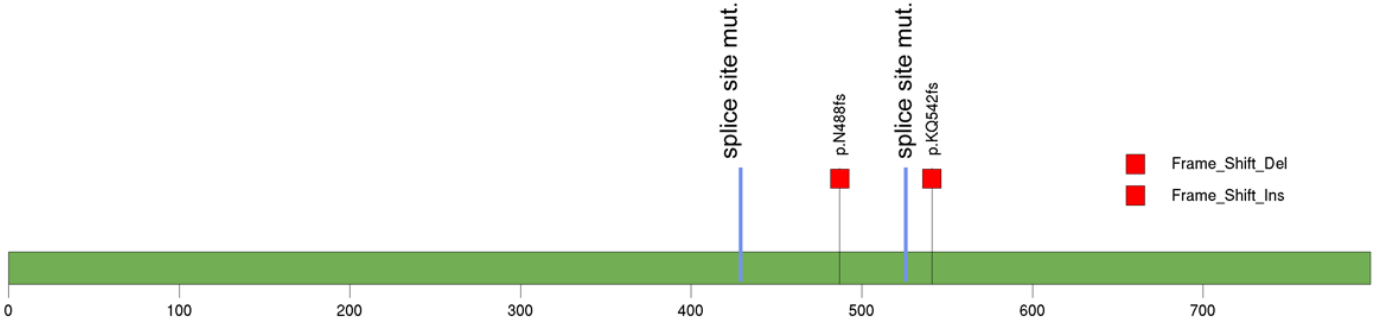

*RET* somatic mutations



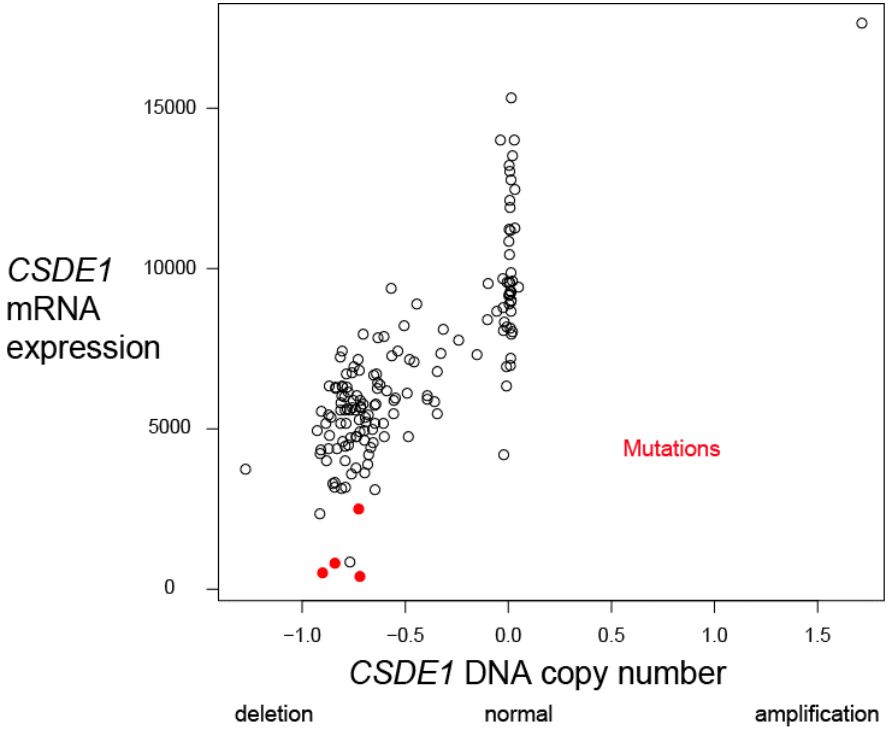

- NF1 and VHL mutations tendencies not different by mutation origin.

# *CSDE1* – new driver gene in Pheo

- Cold Shock Domain Containing E1, RNA-Binding




- *CSDE1* mutant tumors co-occur with DNA copy number deletion and extreme low expression
- Supports loss of function role for *CSDE1*




- Kobyashi et al. (2013) *Neuroscience*: Knock out of *CSDE1* causes irregular neuronal migration in brain development



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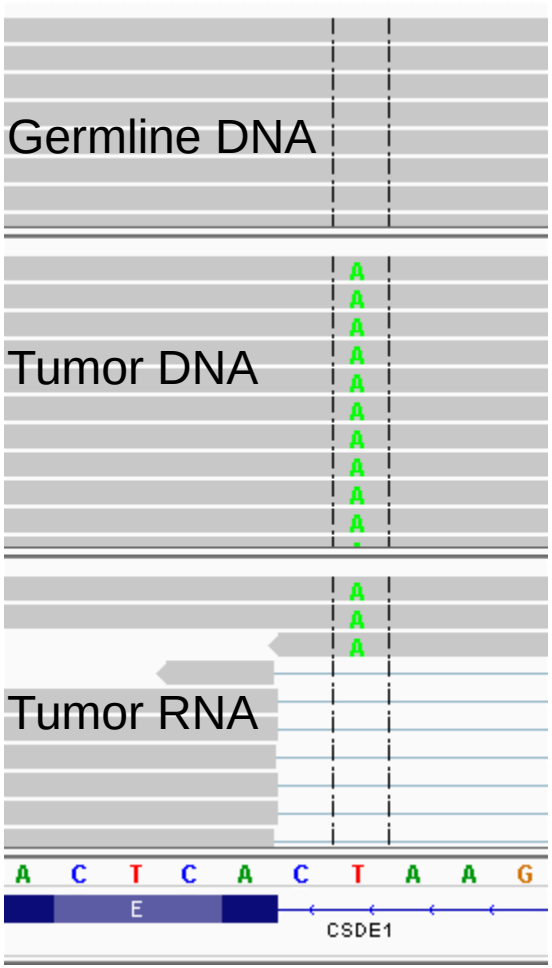
# *CSDE1* Splice Site Mutation causes intron retention

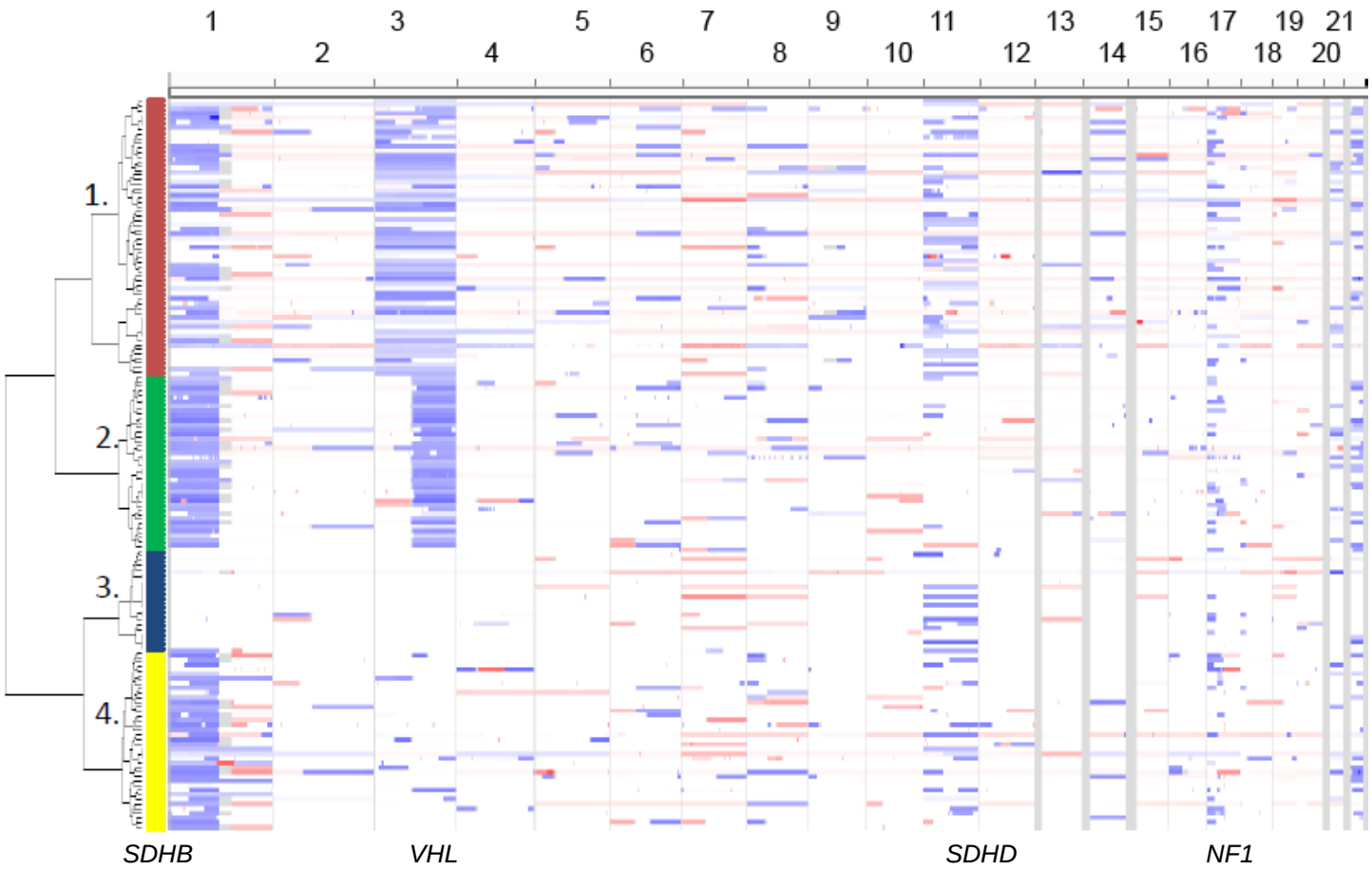



RNA DNA UNCeqR

(Wilkerson et al. 2014 Nucleic Acids Research)

- Tumor mRNA transcripts contain mutation in acceptor splice site, and show intron retention

# Somatic Copy Number Clusters



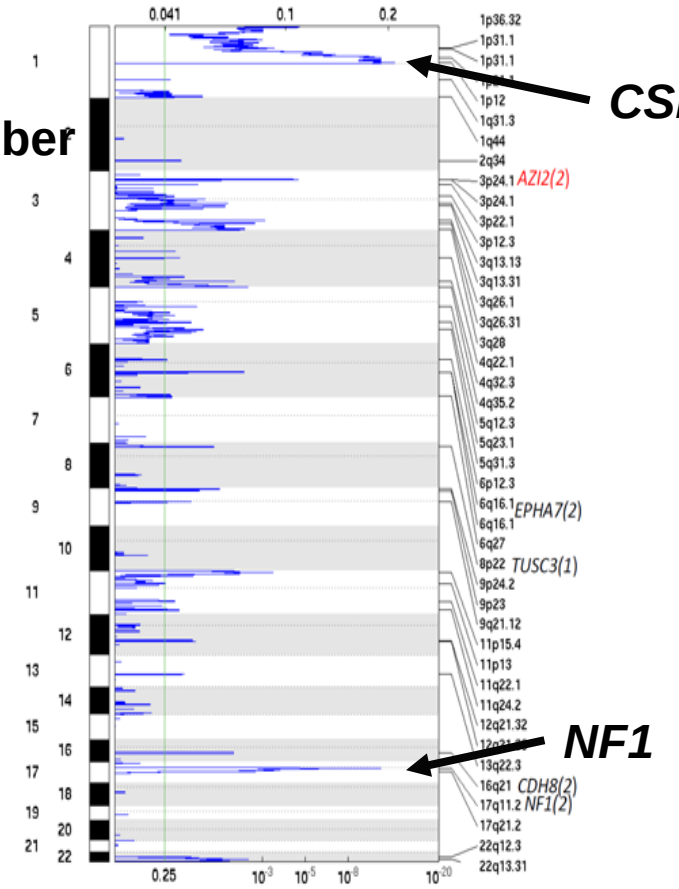

- Deletion regions associated with inherited susceptibility gene

(Brad Murray)

# Focal DNA copy number alterations

- Analysis of recurrent somatic copy number alterations (GISTIC)

**Focal copy number deletions**



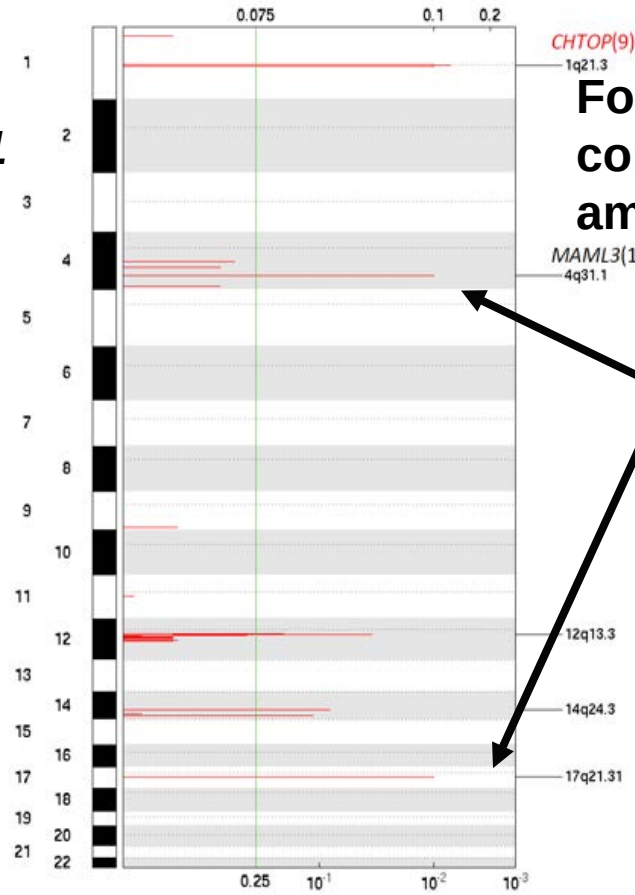

***CSDE1***

***NF1***

Statistical significance

increasing

**Focal copy number amplifications**




4q31, 17q21

Statistical significance

Brad Murray



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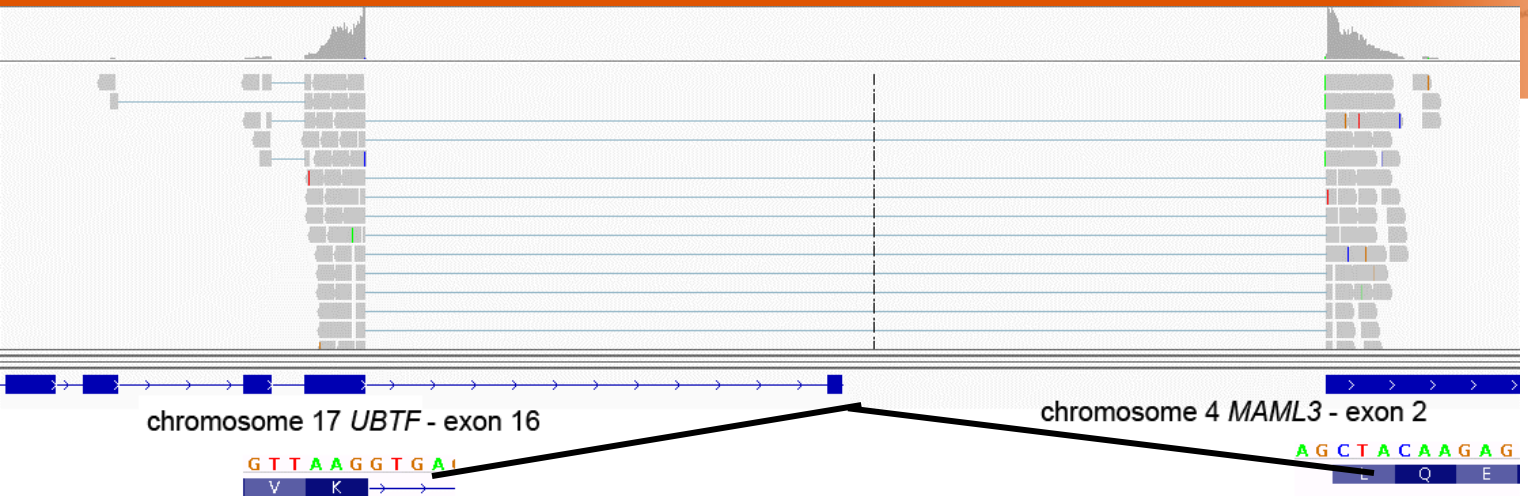
# Novel recurrent *MAML3* fusion gene

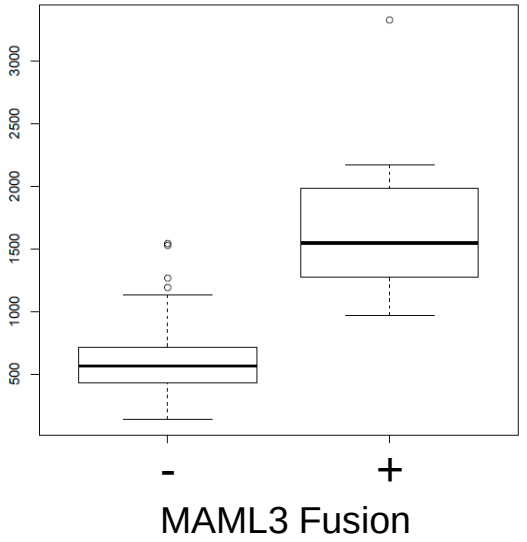



Matt Wilkerson
Stuart Jeffrys

Mapsplice alignments (Wang, Lui, Prins et al. 2010 Nucleic Acids Research)

- **7 cases with *UBTF-MAML3* fusion**
  - *UBTF* – "upstream binding TF"
  - *MAML3* – "mastermind-like 3"
  - 2 fusion isoforms: UBTF exon 14, UBTF exon 16
- 1 case with *TCF4* - *MAML3* fusion
  - TCF4 – "transcription factor 4"
- *MAML3* is highly over-expressed in positive cases

MAML3 mRNA expression



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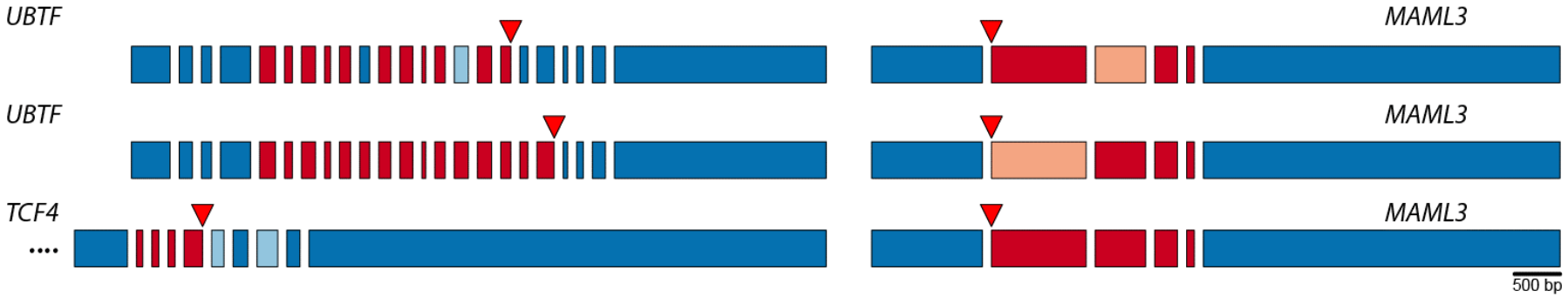
# Novel *MAML3* Fusion Gene

Stuart Jeffrys (UNC)

- *Exonic Expression Analysis*
  - Supports fusion gene expression pattern
  - Promoter of UBTF or TCF4 driving over-expression of MAML3




Legend:

Intra-gene over-expression under-expression

# Characterization of *MAML3* Fusion

- *MAML3* Known to be NOTCH co-activator, but fusion gene lacks NOTCH binding site
  - Similar to PAX3-MAML3 fusion in sinonasal sarcoma (Wang et al. 2014 Nature Genetics)
- Analysis by platform:

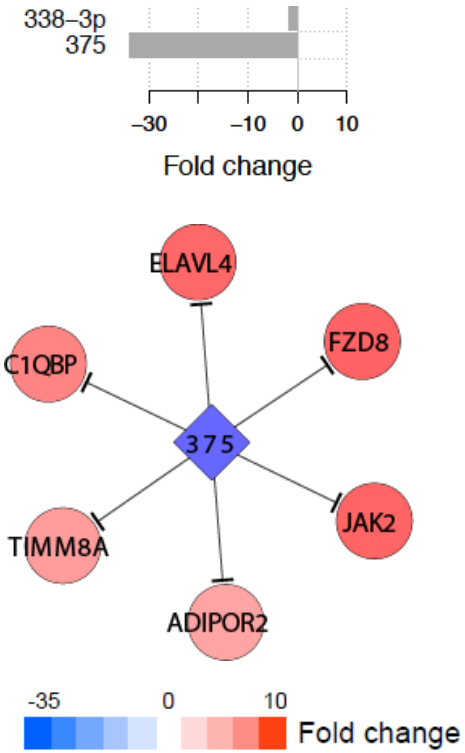
## mRNA expression

GO:0007267~cell-cell signaling
GO:0007268~synaptic transmission
GO:0016055~Wnt receptor signaling pathway
GO:0019226~transmission of nerve impulse
GO:0030032~lamellipodium assembly
GO:0046903~secretion

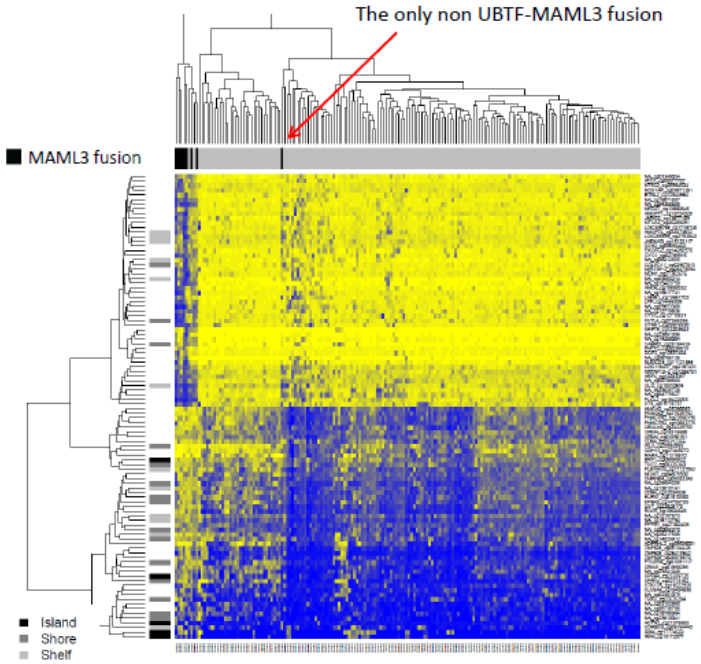
DVL3, FZD3/7/8, WNT4/5B/7B/88

- MAML3 fusion tumors have activated Wnt signaling

## miRNA expression




## DNA Methylation



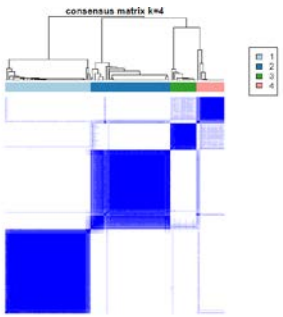

Hypomethylated probes includes FZD3 and WNT's

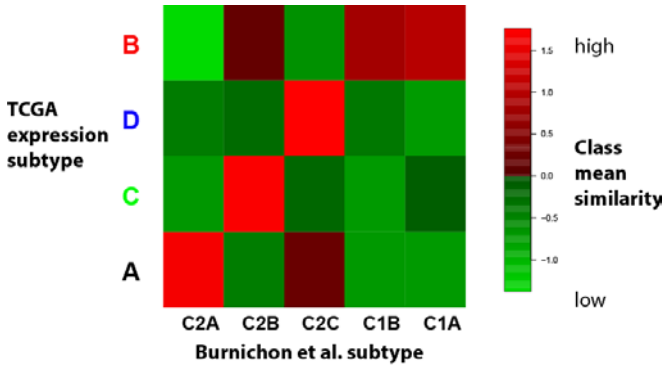


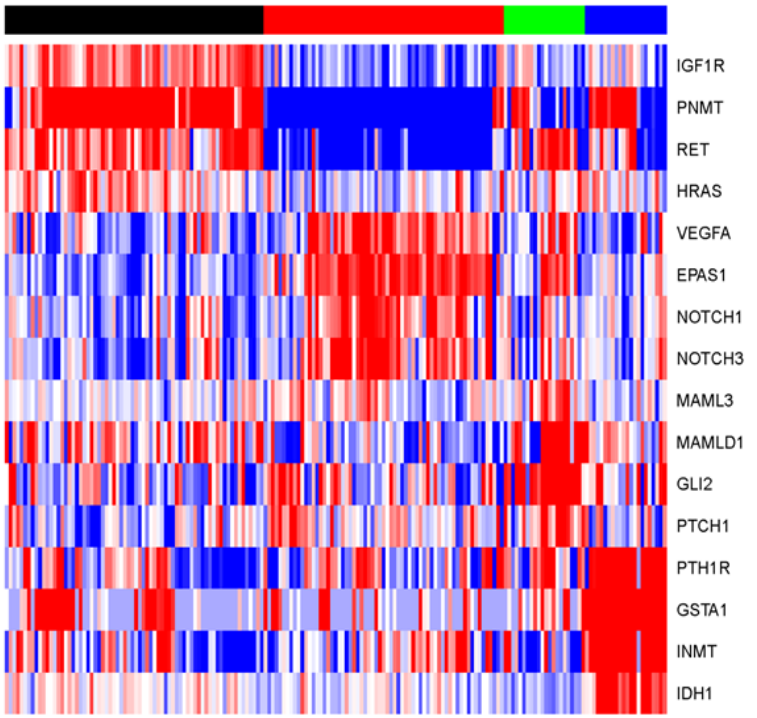
Vonn Walter (UNC) Gordon Robertson (BCCA) Ludmila Danilova (JHU)

# Pheo classified into 4 expression subtypes

- Detected 4 unsupervised expression subtypes
  - (Wilkerson et al Bioinformatics. 2010 Jun 15;26(12):1572-3)
  - Statistically significant (Sigclust on all pairs $P < 0.05$)
- Major patterns of mRNA expression in Pheo
- Subtype mean profiles correspond to published subtypes
  - Burnichon et al. Hum Mol Genet. 2011 Oct 15;20(20):3974-85
- The subtypes are reproducible molecular classes across cohorts



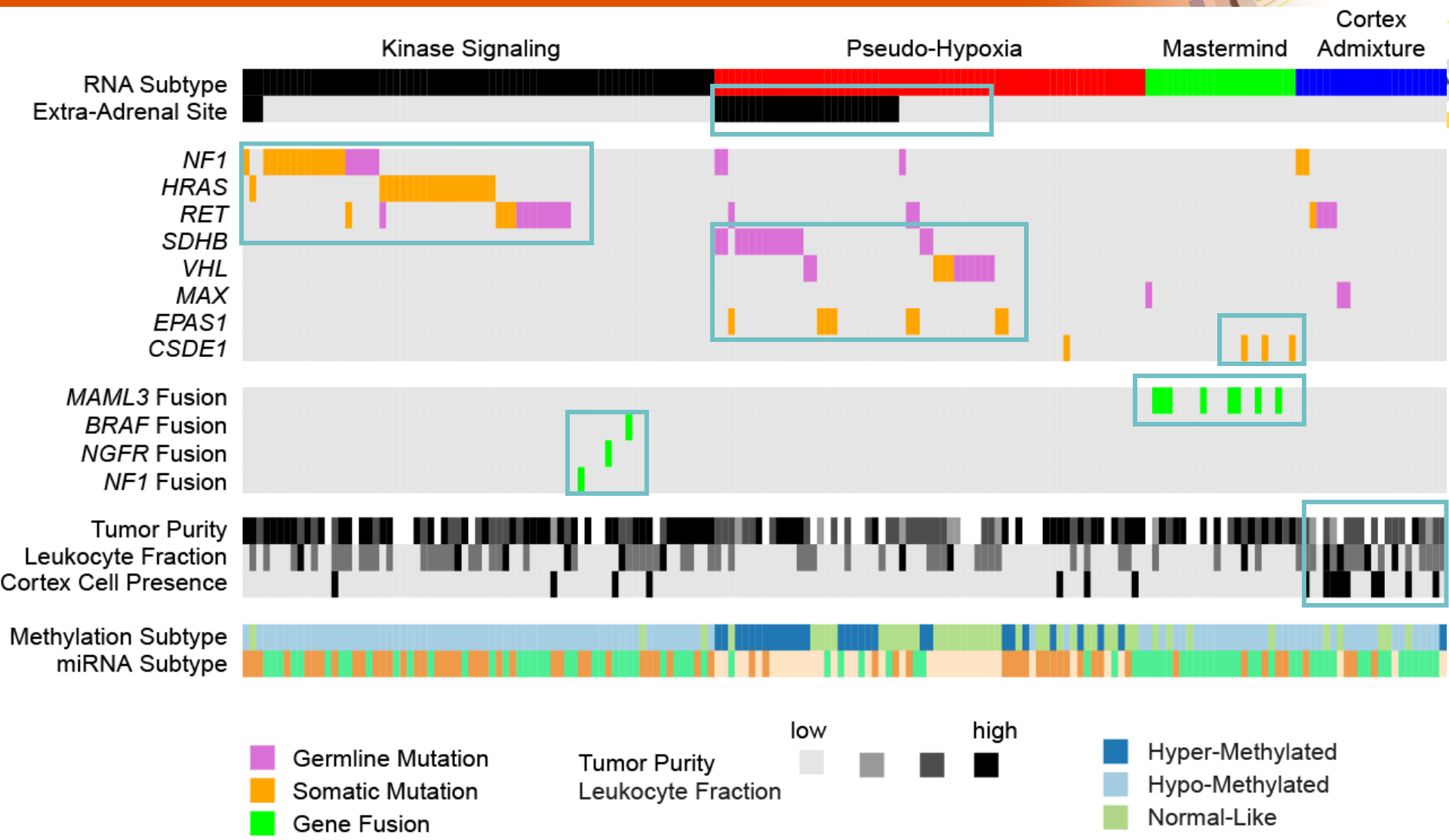






Vonn Walter

# Differential molecular pathogenesis by mRNA expression subtypes




Vonn Walter



*P* < 0.05 on each feature with subtype, except singleton fusions

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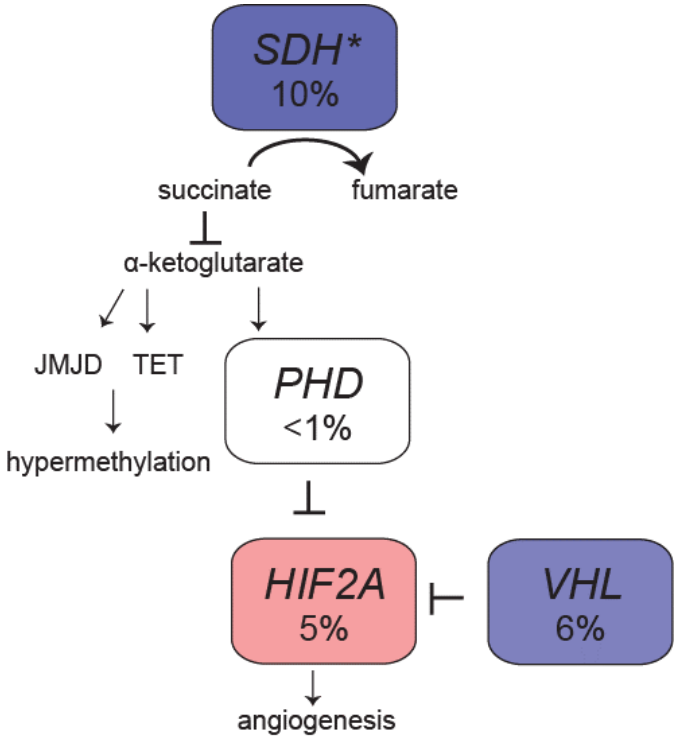
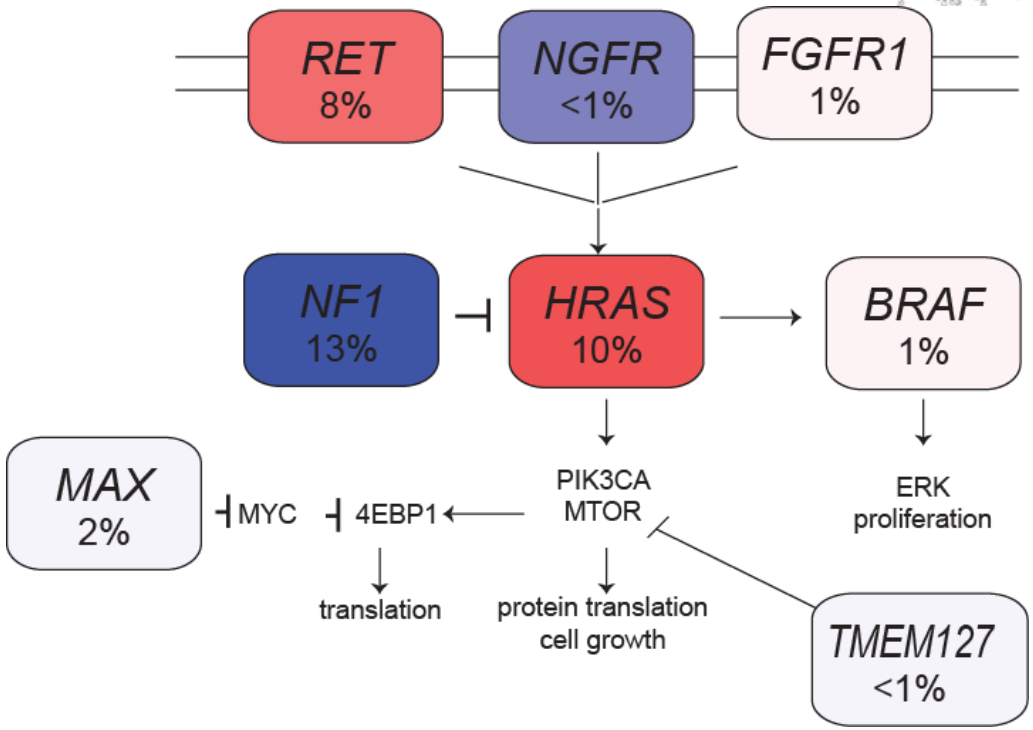
# Distinct pathways

Psuedohypoxia




Highly specific to
Pseudohypoxia subtype

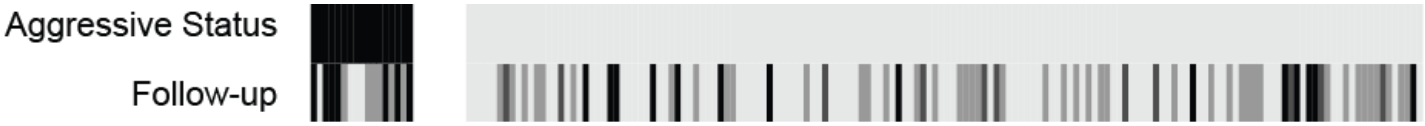
PIK3-AKT & MAPK pathways




Highly specific to
Kinase signaling subtype

# Genomic features of aggressive disease

Lauren Fishbein
Vonn Walter
Kate Nathanson
Tobias Else
Karel Pacak
Matt Wilkerson



# Summary of the new discoveries of the TCGA Pheo Study

1. ~65 % cases have a driving germline or somatic mutation.

2. First recurrent fusion gene in Pheo (*MAML3*)
   - Associates with clinically aggressive disease
   - Found in one expression subtype of sporadic Pheo
   - Overexpresses Wnt signaling pathway

3. First reports of other alterations
   - *CSDE1* somatic mutations
   - Fusion genes in (*NGFR, BRAF, NF1)*

# Acknowledgements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