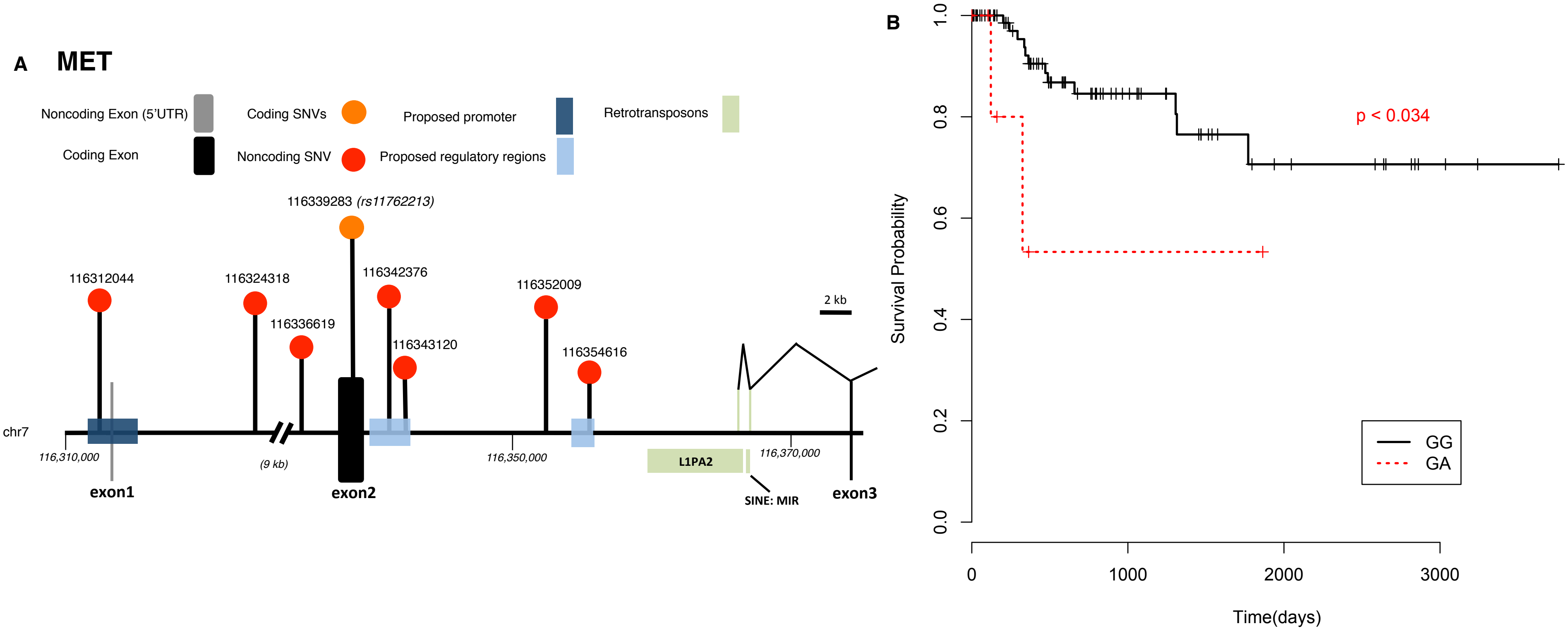
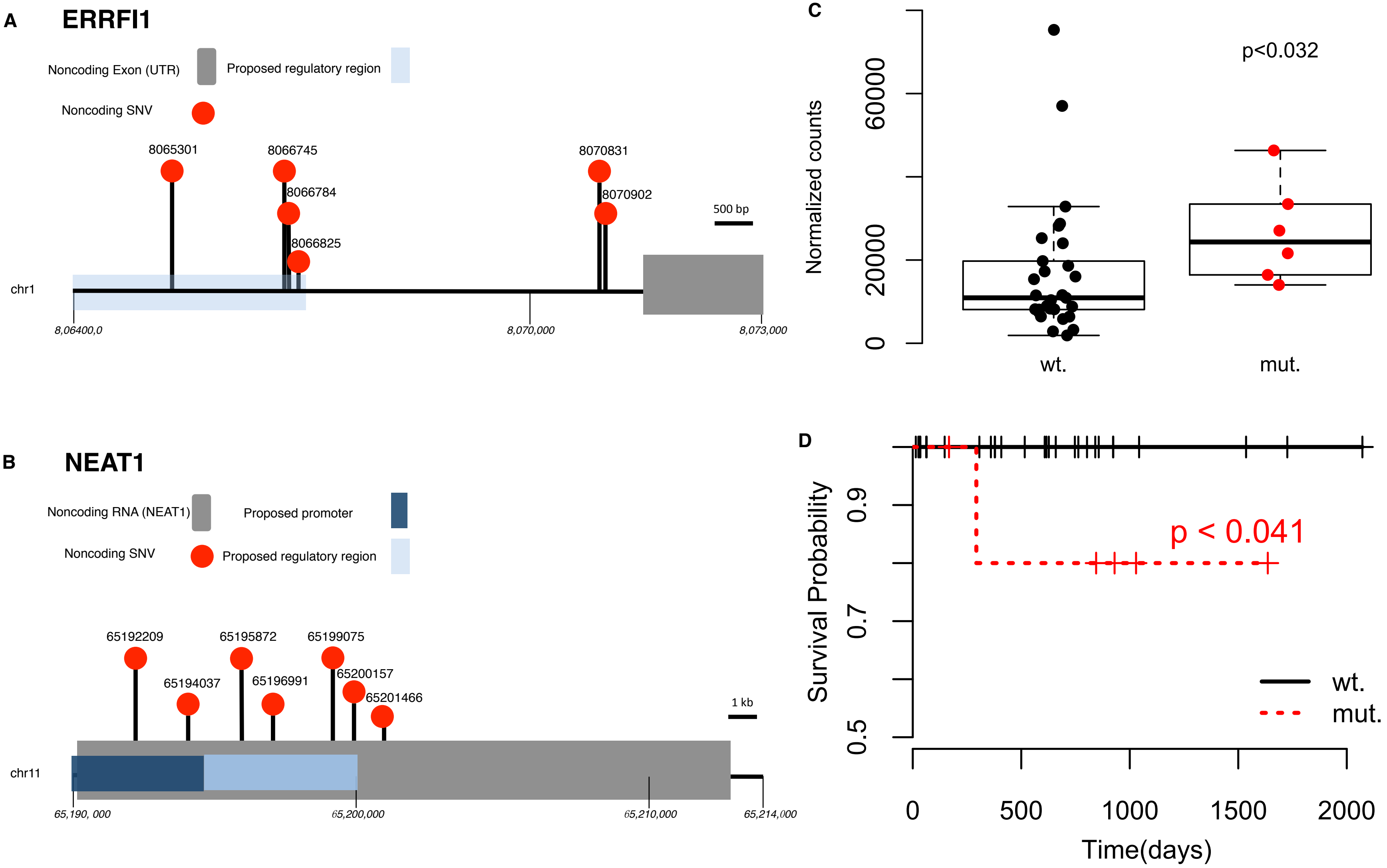
What’s new

- Figure re-plot
- SV comparison
- New DHS/RepliSEQ
- Evolution tree

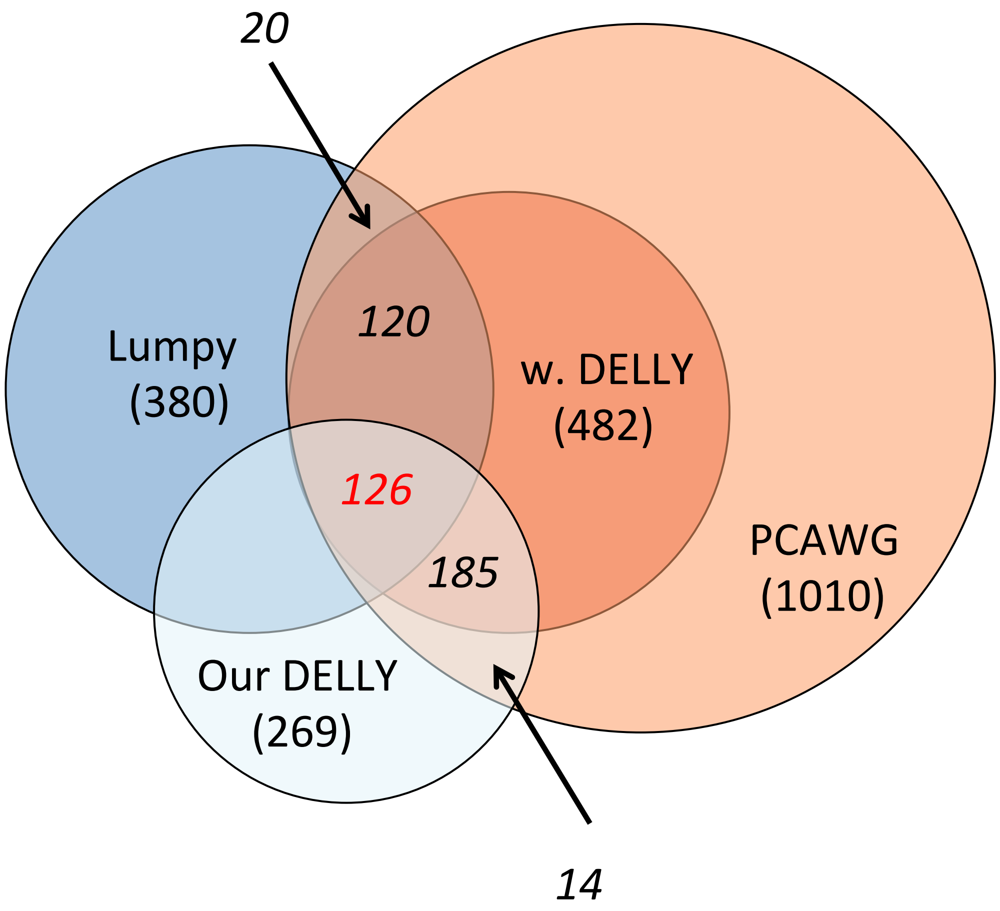

A
MET
Noncoding Exon (5'UTR)
Coding Exon
Coding SNVs
Noncoding SNV
Proposed promoter
Proposed regulatory regions
Retrotransposons
116339283 (rs11762213)
116312044
116324318
116336619
116342376
116343120
116352009
116354616
2 kb
chr7
116,310,000
(9 kb)
116,350,000
116,370,000
L1PA2
SINE: MIR
exon1
exon2
exon3
B
Survival Probability
0.0
0.2
0.4
0.6
0.8
1.0
Time(days)
0
1000
2000
3000
p < 0.034
GG
GA


A
ERRFI1
Noncoding Exon (UTR)
Proposed regulatory region
Noncoding SNV
8065301
8066745
8066784
8066825
8070831
8070902
500 bp
chr1
8,06400,0
8,070,000
8,073,000
B
NEAT1
Noncoding RNA (NEAT1)
Proposed promoter
Noncoding SNV
Proposed regulatory region
65192209
65194037
65195872
65196991
65199075
65200157
65201466
1 kb
chr11
65,190, 000
65,200,000
65,210,000
65,214,000
C
Normalized counts
0
20000
60000
p<0.032
wt.
mut.
D
Survival Probability
0.5
0.7
0.9
p < 0.041
wt.
mut.
0
500
1000
1500
2000
Time(days)

SV comparison

- First, why Lumpy/SVscore?
  - Our strength at bkpts (as to aCGH)
    - Overlapping bkpts with functional regions
    - What about bkpt labeled as IMPRECISE
  - Lumpy/SVscore gives the right normalization
    - Normalized CADD scores around bkpts
    - Report intervals as well
  - Issues: Quality & SVTyper
    - The genotyper works weirdly

SV comparison



This is on 32 samples
(*we called SV from 35*)

Criteria:

1. *0.5* reciprocal overlap
2. Matched sample
3. Matched SV CLASS

Only get *126* SVs if overlapping DELLY with Lumpy using 32 samples

SV comparison

- If we think PCAWG DELLY is the ground truth
  - Other methods rely on assembly…different searching space
    - complicated SV events produce many bkpts
  - Not a fair comparison

	#SVs	FN	FP	Addl. catch
Lumpy	380	71%	63.2%	20 (5.26%)
Delly (ours)	269	61.6%	31.2%	14 (5.20%)

Bkpts&interval overlapping

- No overlapping with pRCC MutSig genes (~10)
- COSMIC genes

	bkpts
1	AFF3
1	AKAP9
1	ATRX
1	CDK6
1	CDKN2A
1	CLP1
1	CREBBP
1	ERCC2
1	LRP1B
1	MKL1
1	MLLT10
1	POLE
1	POT1
1	SND1
1	SOX2-OT
1	STAT5B
1	STK11
1	THRAP3
2	SPEN

- Three overlapping with pRCC MutSig
  - Extensive SVs in TCGA-B9-4116
  - DEL of STAG2
  - A large INV involves NFE2L2

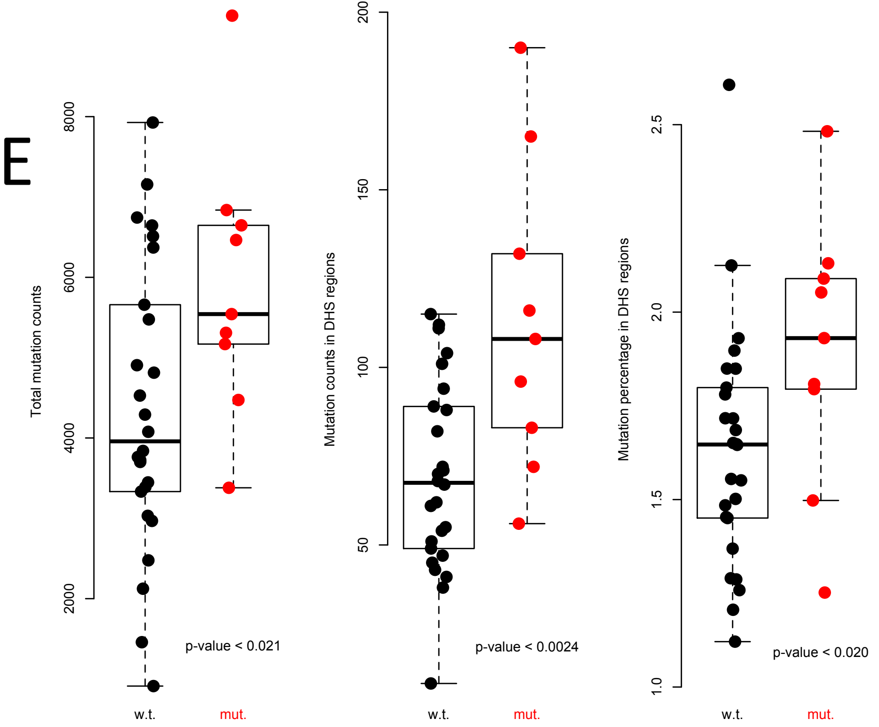
SV overlapping

- Some interesting COSMIC cases
  - 3 CDKN3A (confirmed NEJM 3/5 cases)
  - 2 SDHB deletion (interacts with FH and SDHA)
  - 1 EGFR duplication (pRCC responses to TKIs)
  - 1 HIF1A duplication (inhibited by VHL;)
  - 1 polyE bkpts (same case)
  - 1 DNMT3A deletion (affect methylation)
  - 1 MALAT1 *deletion*
  - 1 HGF *deletion (ligand for MET)*

1	POLE2
1	DNMT3A
1	HIF1A
1	IDH1
1	MALAT1
2	EGFR
2	SDHB
3	CDKN2A
1	HGF

New DHS scheme

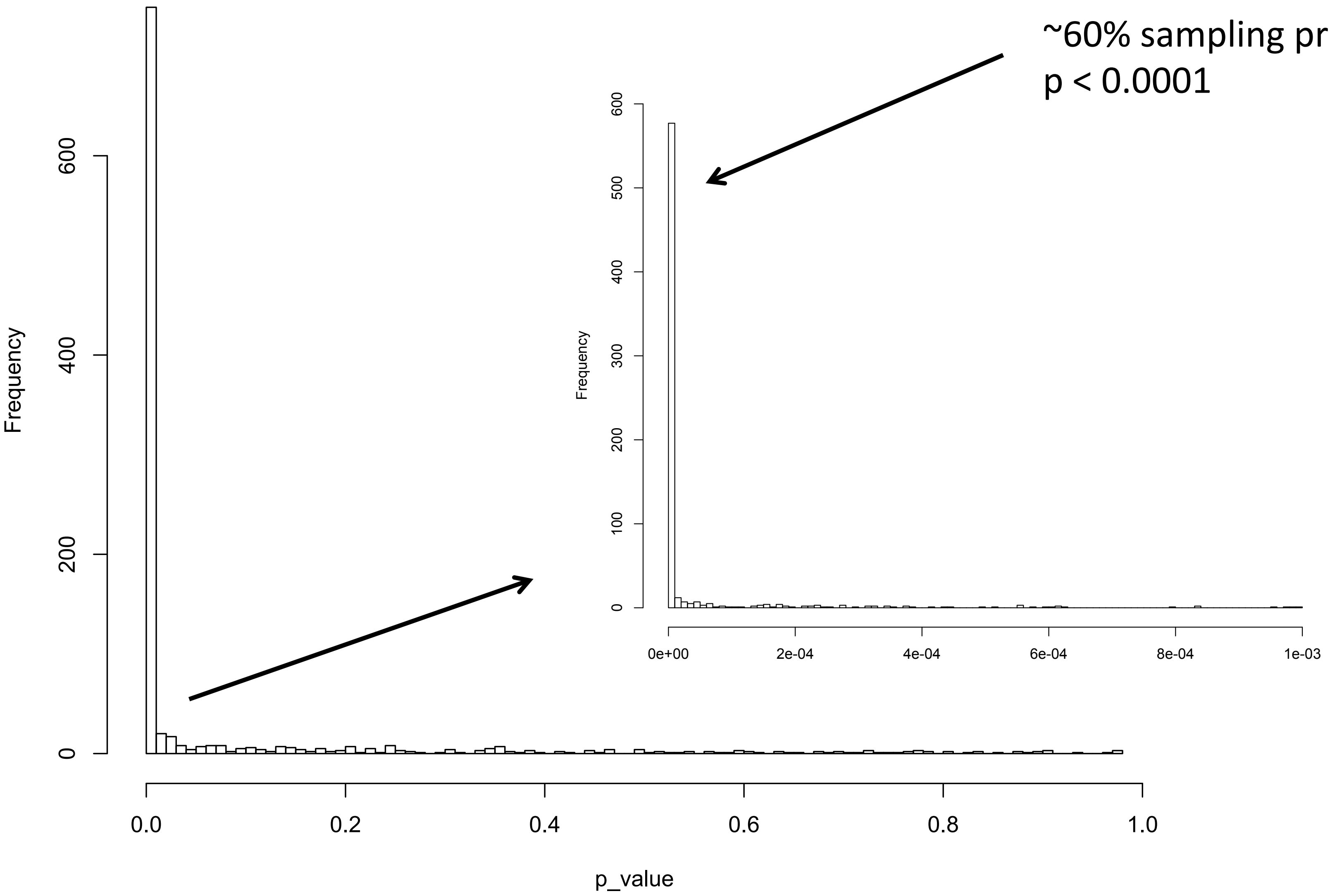
- Pulled fetal kidney cortex DHS from Roadmap
- 11 samples from different fetuses
  - Ultra-conservative: take the overlap of all samples
  - DHS percentage roughly matches ENCODE HEK293 narrowpeak (previously used)



RepliSEQ

- Newly defined CR-genes
  - Overlapping NEJM spreadsheet(pRCC-related) genes with CR&SWI/SNF pathway gene list
- Conservative RT signals (*Ref: NGen.*)
  - Taken median from 11 ENCODE cell lines
- Prudent adapted KS test (subsampling)
  - Q: does the RT dist. of SNVs from CR$^{mut.}$ samples differ from CR$^{wt.}$ samples
  - Randomly shuffle labels (9 v.s. 26) for 1,000 times to generate imperial TS distribution

Why we need subsampling

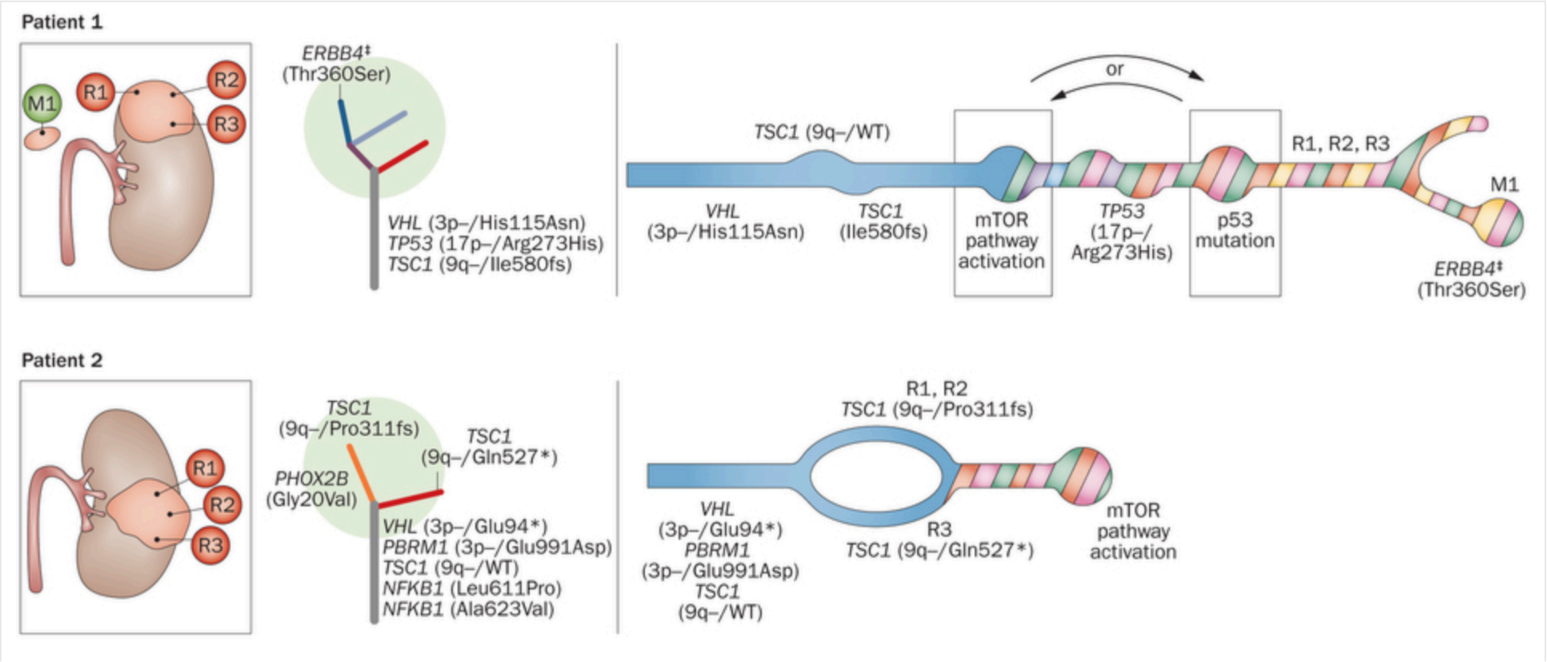


~60% sampling produce
p < 0.0001

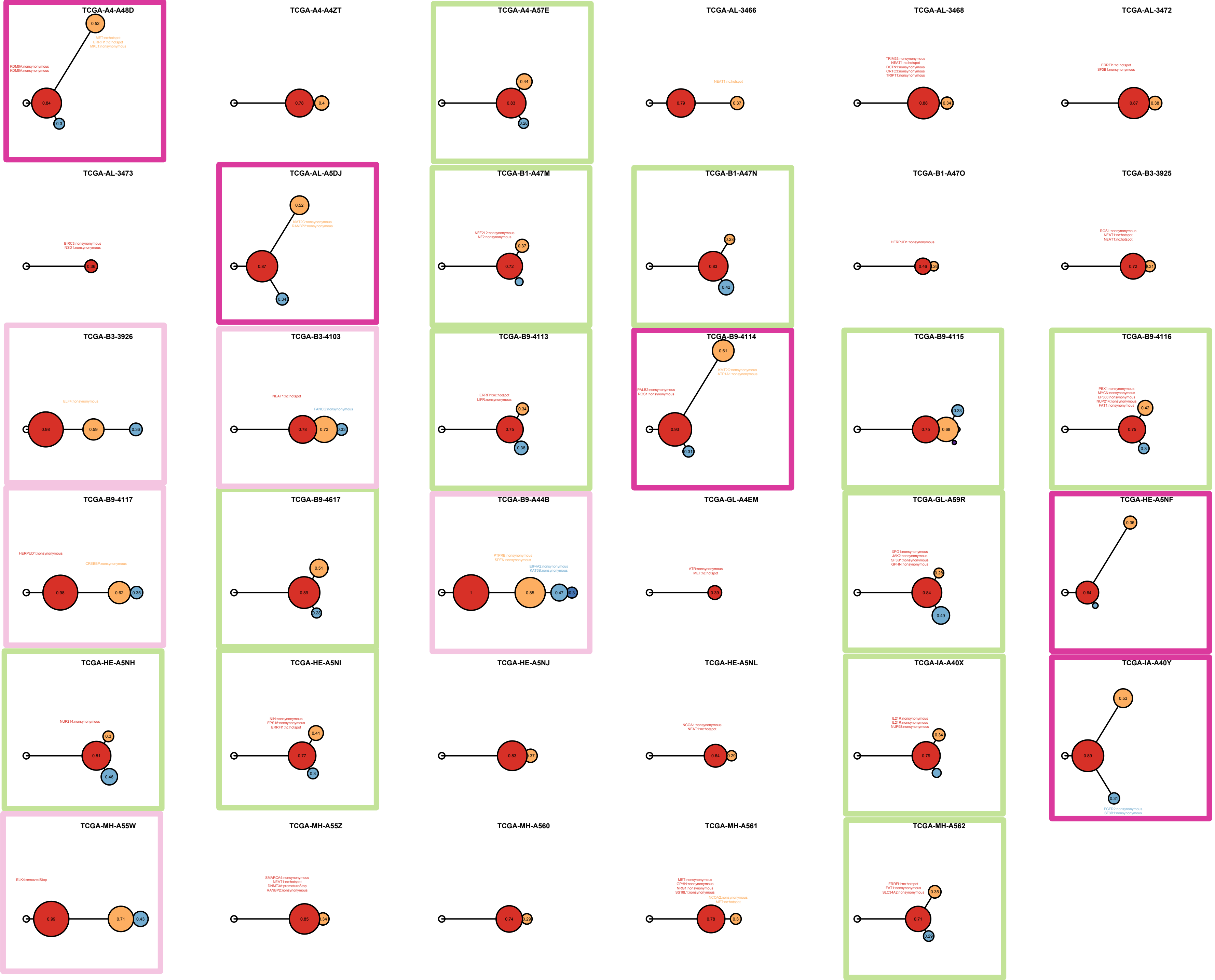
KS tests w/ subsampling: Issues

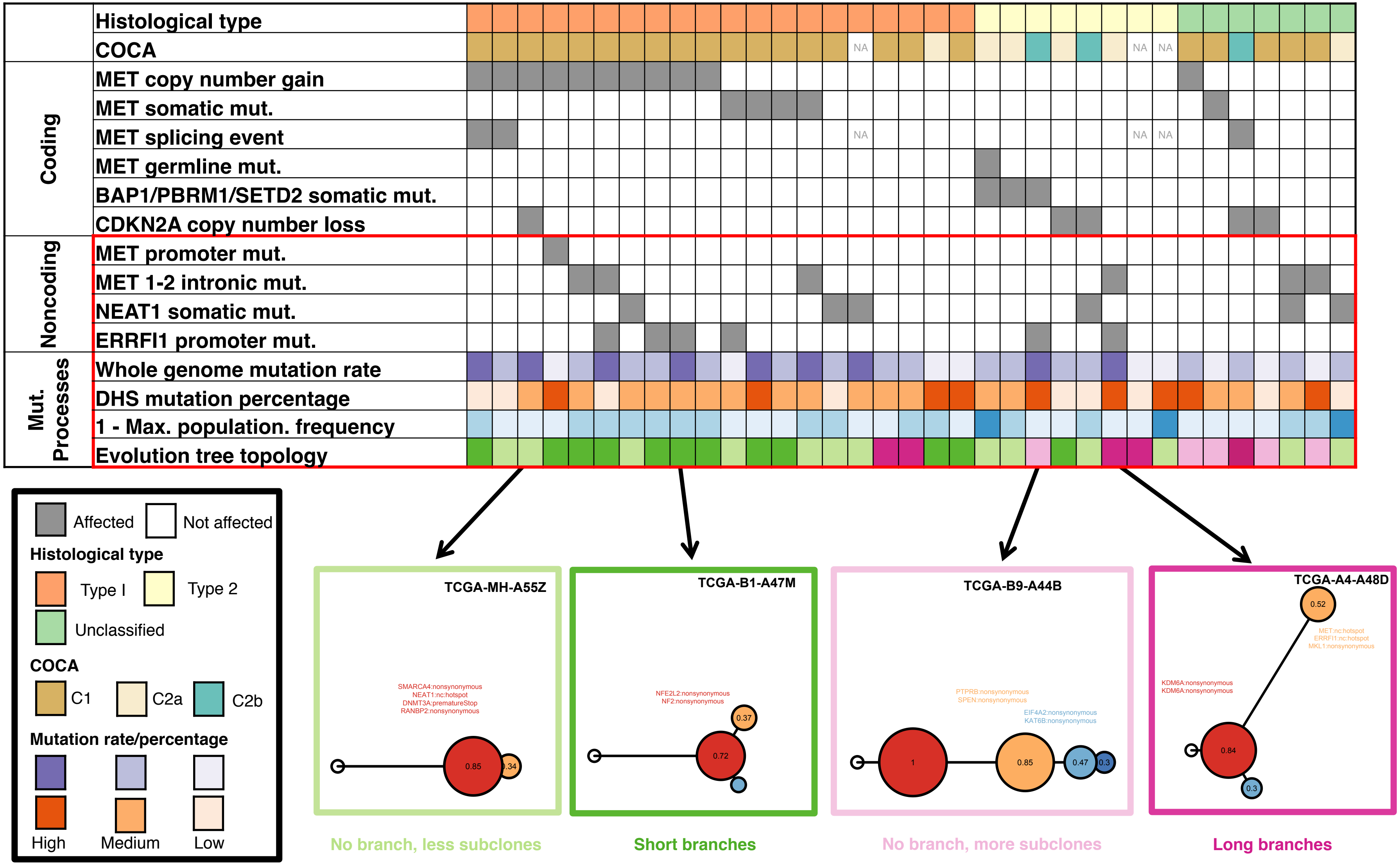
- A. p-value below limits
  - Our test produces p < 2.2e-16 (which becomes 0)
  - However, we observed 26% such cases
- B. test power not uniform
  - SNVs in each sample varies
  - Makes test statistic (D) not directly comparable
  - Worse: power is not uniform!

Evolution tree



- Less interesting because:
  - Single punch from each patient
  - Not ultra-deep sequence
  - Masked all CNV + MAF>0.6


TCGA-A4-A48D
0.52
MET:nc:hotspot
ERRFI1:nc:hotspot
MKL1:nonsynonymous
KDM6A:nonsynonymous
KDM6A:nonsynonymous
0.84
0.3
TCGA-A4-A4ZT
0.78
0.4
TCGA-A4-A57E
0.44
0.83
0.28
TCGA-AL-3466
NEAT1:nc:hotspot
0.79
0.37
TCGA-AL-3468
TRIM33:nonsynonymous
NEAT1:nc:hotspot
DCTN1:nonsynonymous
CRTC3:nonsynonymous
TRIP11:nonsynonymous
0.88
0.34
TCGA-AL-3472
ERRFI1:nc:hotspot
SF3B1:nonsynonymous
0.87
0.38
TCGA-AL-3473
BIRC3:nonsynonymous
NSD1:nonsynonymous
0.36
TCGA-AL-A5DJ
0.52
KMT2C:nonsynonymous
RANBP2:nonsynonymous
0.87
0.34
TCGA-B1-A47M
NFE2L2:nonsynonymous
NF2:nonsynonymous
0.37
0.72
TCGA-B1-A47N
0.28
0.83
0.42
TCGA-B1-A47O
HERPUD1:nonsynonymous
0.46
.26
TCGA-B3-3925
ROS1:nonsynonymous
NEAT1:nc:hotspot
NEAT1:nc:hotspot
0.72
0.31
TCGA-B3-3926
ELF4:nonsynonymous
0.98
0.59
0.36
TCGA-B3-4103
NEAT1:nc:hotspot
FANCG:nonsynonymous
0.78
0.73
0.33
TCGA-B9-4113
ERRFI1:nc:hotspot
LIFR:nonsynonymous
0.34
0.75
0.38
TCGA-B9-4114
0.61
KMT2C:nonsynonymous
ATP1A1:nonsynonymous
PALB2:nonsynonymous
ROS1:nonsynonymous
0.93
0.31
TCGA-B9-4115
0.33
0.75
0.68
TCGA-B9-4116
PBX1:nonsynonymous
MYCN:nonsynonymous
EP300:nonsynonymous
NUP214:nonsynonymous
FAT1:nonsynonymous
0.42
0.75
0.3
TCGA-B9-4117
HERPUD1:nonsynonymous
CREBBP:nonsynonymous
0.98
0.62
0.35
TCGA-B9-4617
0.51
0.89
0.28
TCGA-B9-A44B
PTPRB:nonsynonymous
SPEN:nonsynonymous
EIF4A2:nonsynonymous
KAT6B:nonsynonymous
1
0.85
0.47
0.3
TCGA-GL-A4EM
ATR:nonsynonymous
MET:nc:hotspot
0.39
TCGA-GL-A59R
XPO1:nonsynonymous
JAK2:nonsynonymous
SF3B1:nonsynonymous
GPHN:nonsynonymous
0.28
0.84
0.49
TCGA-HE-A5NF
0.36
0.64
TCGA-HE-A5NH
NUP214:nonsynonymous
0.3
0.81
0.46
TCGA-HE-A5NI
NIN:nonsynonymous
EPS15:nonsynonymous
ERRFI1:nc:hotspot
0.41
0.77
0.3
TCGA-HE-A5NJ
0.83
.37
TCGA-HE-A5NL
NCOA1:nonsynonymous
NEAT1:nc:hotspot
0.64
0.29
TCGA-IA-A40X
IL21R:nonsynonymous
IL21R:nonsynonymous
NUP98:nonsynonymous
0.34
0.79
TCGA-IA-A40Y
0.53
0.89
0.31
FGFR2:nonsynonymous
SF3B1:nonsynonymous
TCGA-MH-A55W
ELK4:removedStop
0.99
0.71
0.43
TCGA-MH-A55Z
SMARCA4:nonsynonymous
NEAT1:nc:hotspot
DNMT3A:prematureStop
RANBP2:nonsynonymous
0.85
.34
TCGA-MH-A560
0.74
0.29
TCGA-MH-A561
MET:nonsynonymous
GPHN:nonsynonymous
NRG1:nonsynonymous
SS18L1:nonsynonymous
NCOA2:nonsynonymous
MET:nc:hotspot
0.78
0.3
TCGA-MH-A562
ERRFI1:nc:hotspot
FAT1:nonsynonymous
SLC34A2:nonsynonymous
0.35
0.71
0.29


Histological type
COCA
NA
NA
NA
Coding
MET copy number gain
MET somatic mut.
MET splicing event
NA
NA
NA
MET germline mut.
BAP1/PBRM1/SETD2 somatic mut.
CDKN2A copy number loss
Noncoding
MET promoter mut.
MET 1-2 intronic mut.
NEAT1 somatic mut.
ERRFI1 promoter mut.
Mut. Processes
Whole genome mutation rate
DHS mutation percentage
1 - Max. population. frequency
Evolution tree topology
Affected
Not affected
Histological type
Type I
Type 2
Unclassified
COCA
C1
C2a
C2b
Mutation rate/percentage
High
Medium
Low
TCGA-MH-A55Z
SMARCA4:nonsynonymous
NEAT1:nc:hotspot
DNMT3A:prematureStop
RANBP2:nonsynonymous
0.85
0.34
No branch, less subclones
TCGA-B1-A47M
NFE2L2:nonsynonymous
NF2:nonsynonymous
0.37
0.72
Short branches
TCGA-B9-A44B
PTPRB:nonsynonymous
SPEN:nonsynonymous
EIF4A2:nonsynonymous
KAT6B:nonsynonymous
1
0.85
0.47
0.3
No branch, more subclones
TCGA-A4-A48D
0.52
MET:nc:hotspot
ERRFI1:nc:hotspot
MKL1:nonsynonymous
KDM6A:nonsynonymous
KDM6A:nonsynonymous
0.84
0.3
Long branches

Paper E thoughts

- What we want to sell
  - A complete "tumor portrait"
  - Completeness on several levels
    - Unprecedentedly large WGS cohort
    - Coding/noncoding/SVs…

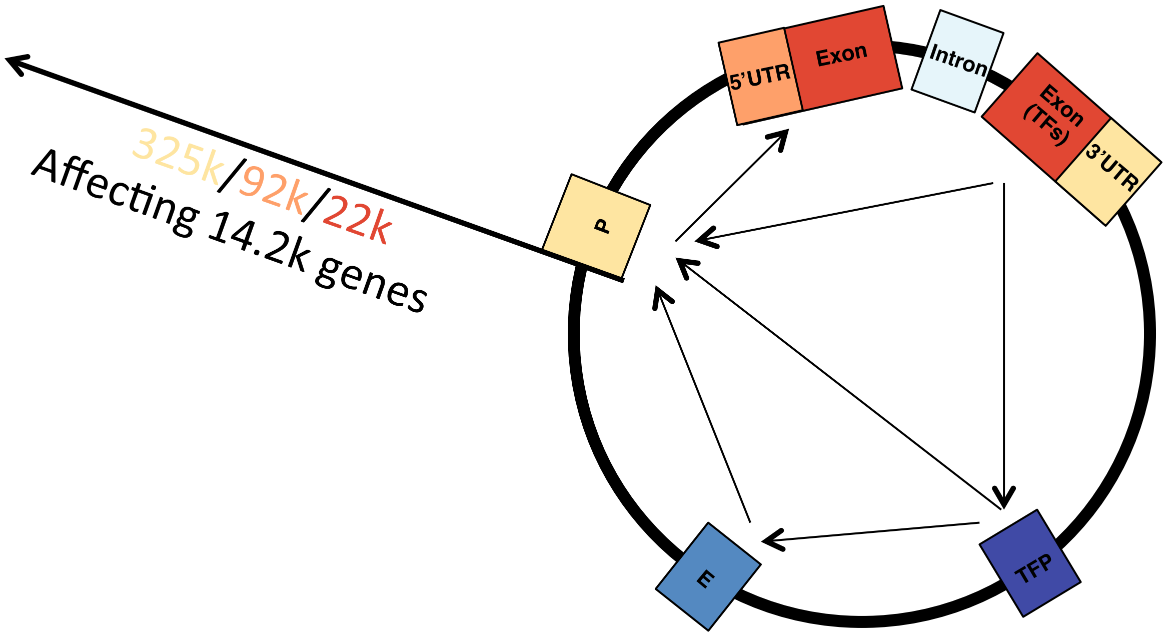
- Fig1: a fancy figure to visualize the deluge of the data
  - Show a gene ("genecreek" or "-circle")

Issues

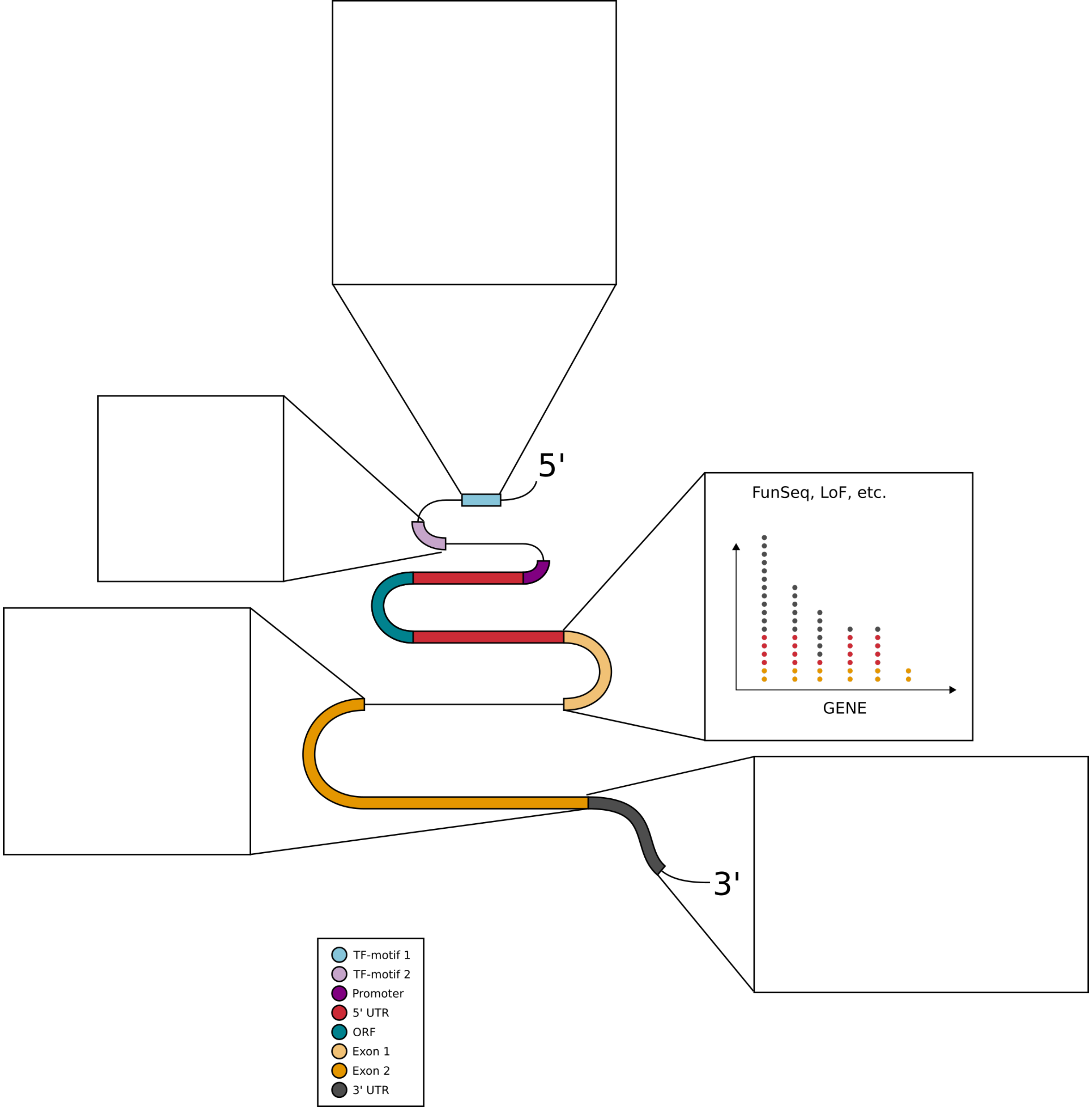
- One sample with 23 EPB41L2 promoter mutation?
  - And 9 has the identical FunSeq score!
  - Annotation issue? This promoter spans >170kb!

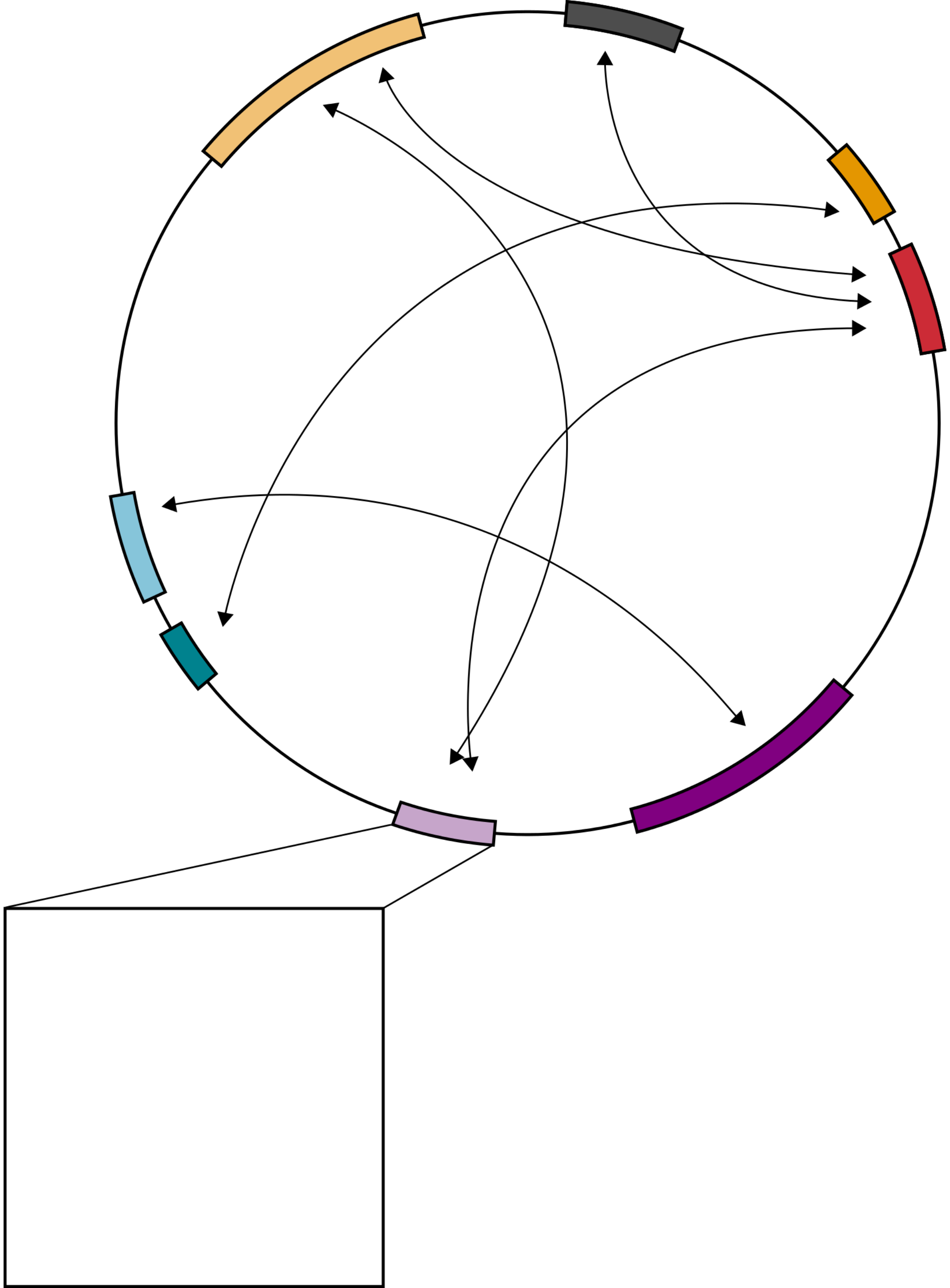
  - However, this is an excluded sample…

  - Still, another included sample has 8…

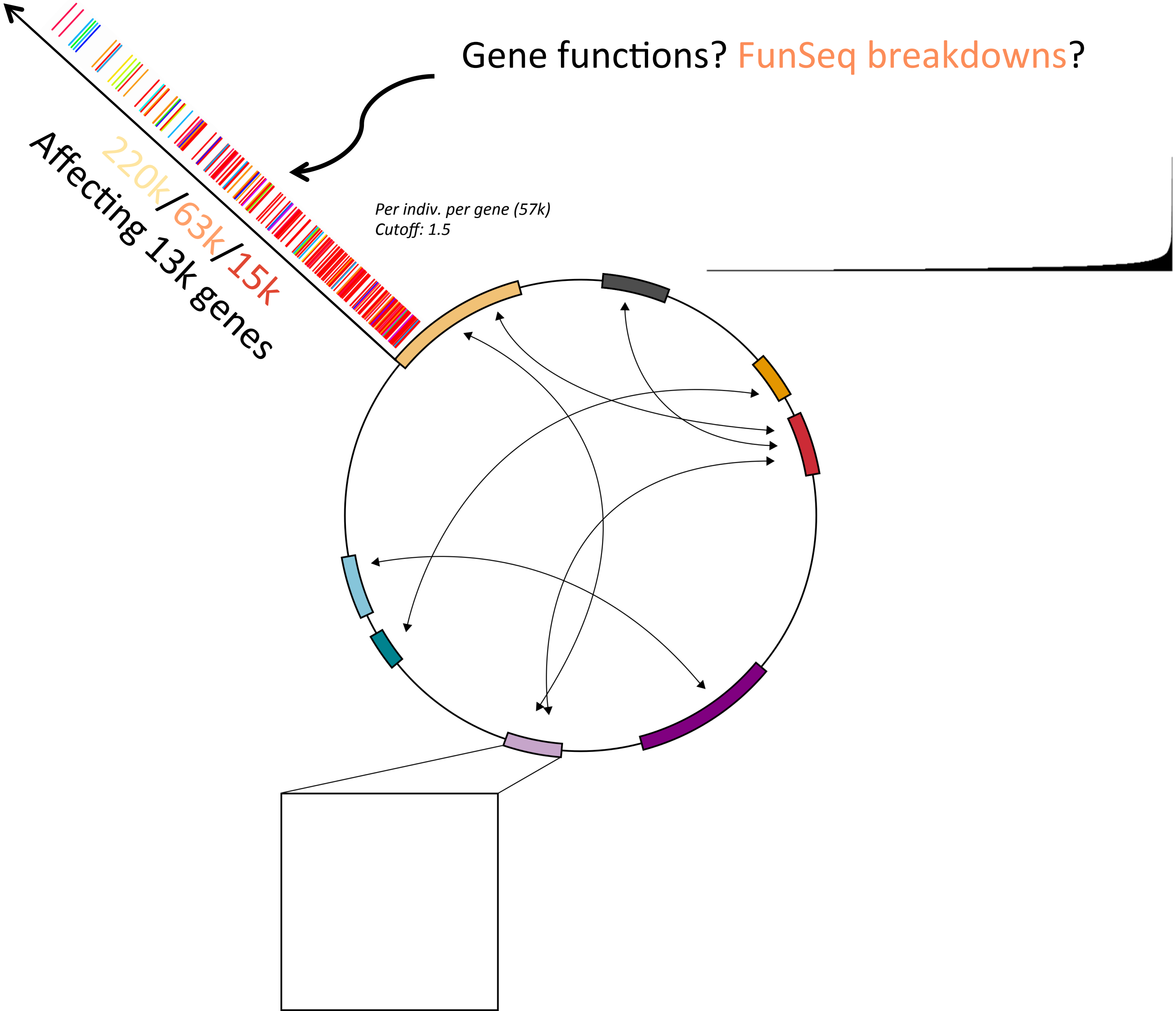


325k/92k/22k
Affecting 14.2k genes


5'
FunSeq, LoF, etc.
GENE
3'
TF-motif 1
TF-motif 2
Promoter
5' UTR
ORF
Exon 1
Exon 2
3' UTR

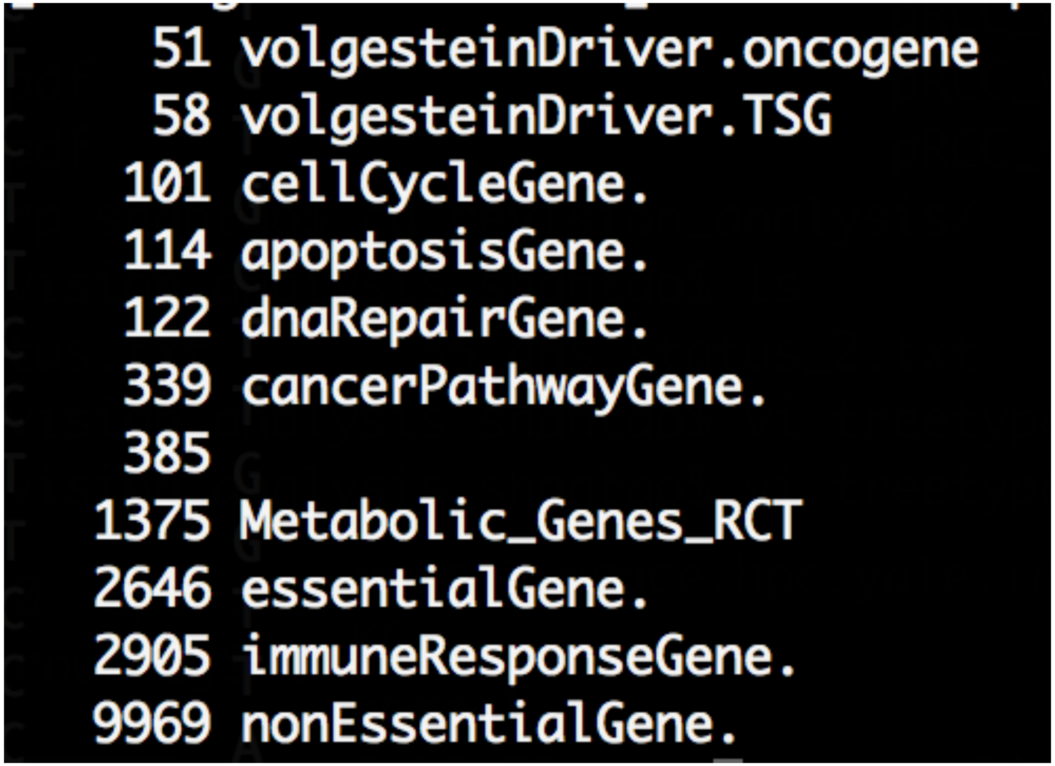


Gene functions? FunSeq breakdowns?



108	TCF4
109	DUSP22
124	TERT
125	SEPT9
154	ZNF595

Annotation



```
  51 volgesteinDriver.oncogene
  58 volgesteinDriver.TSG
 101 cellCycleGene.
 114 apoptosisGene.
 122 dnaRepairGene.
 339 cancerPathwayGene.
 385
1375 Metabolic_Genes_RCT
2646 essentialGene.
2905 immuneResponseGene.
9969 nonEssentialGene.
```

1.Normalize by the #gene
2.Normalize by the length of elements
3.Correct for RT/Trinucleotide context

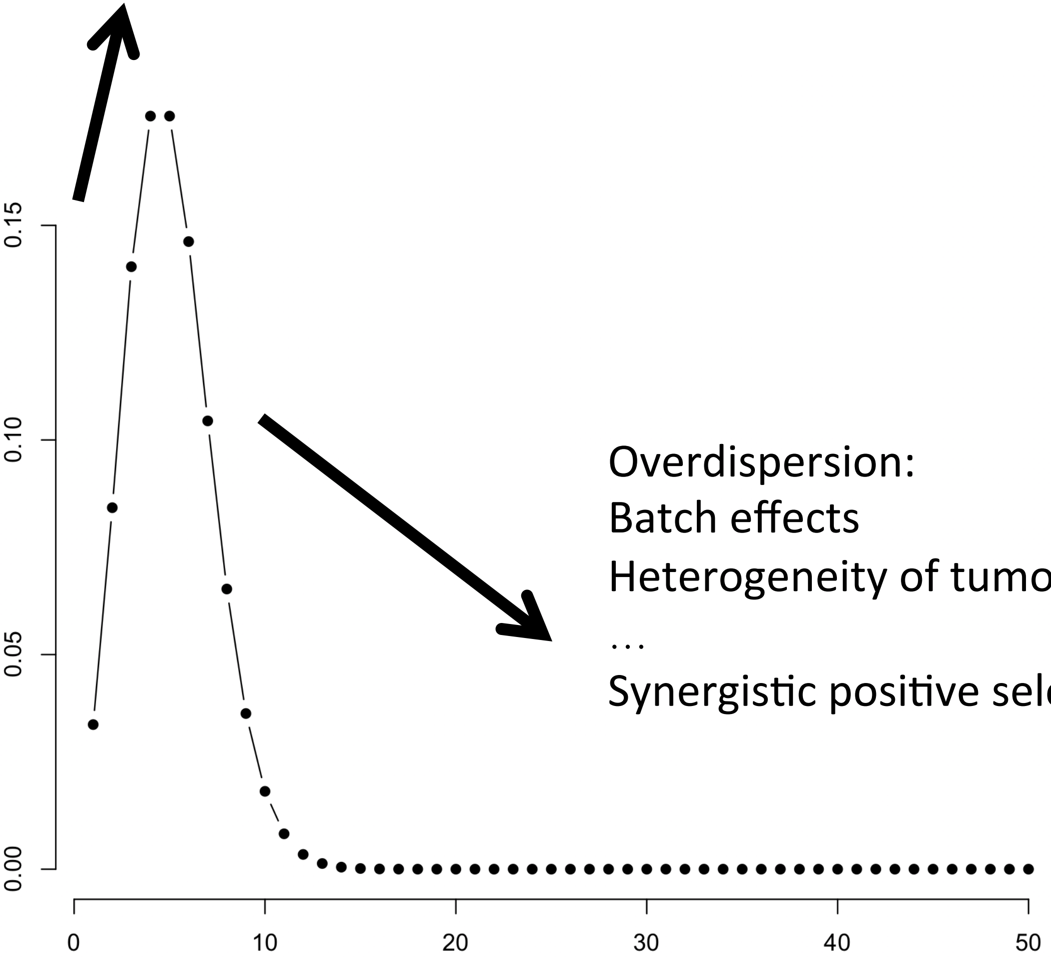
Some high-impact alterations have adverse effects on tumors

No driver/selection…or whatsoever

Hunting underdispersion

Underdispersion:
Synergistic neg. selection
??? (rare)

What violates *i.i.d.* ?



Overdispersion:
Batch effects
Heterogeneity of tumors
…
Synergistic positive selection

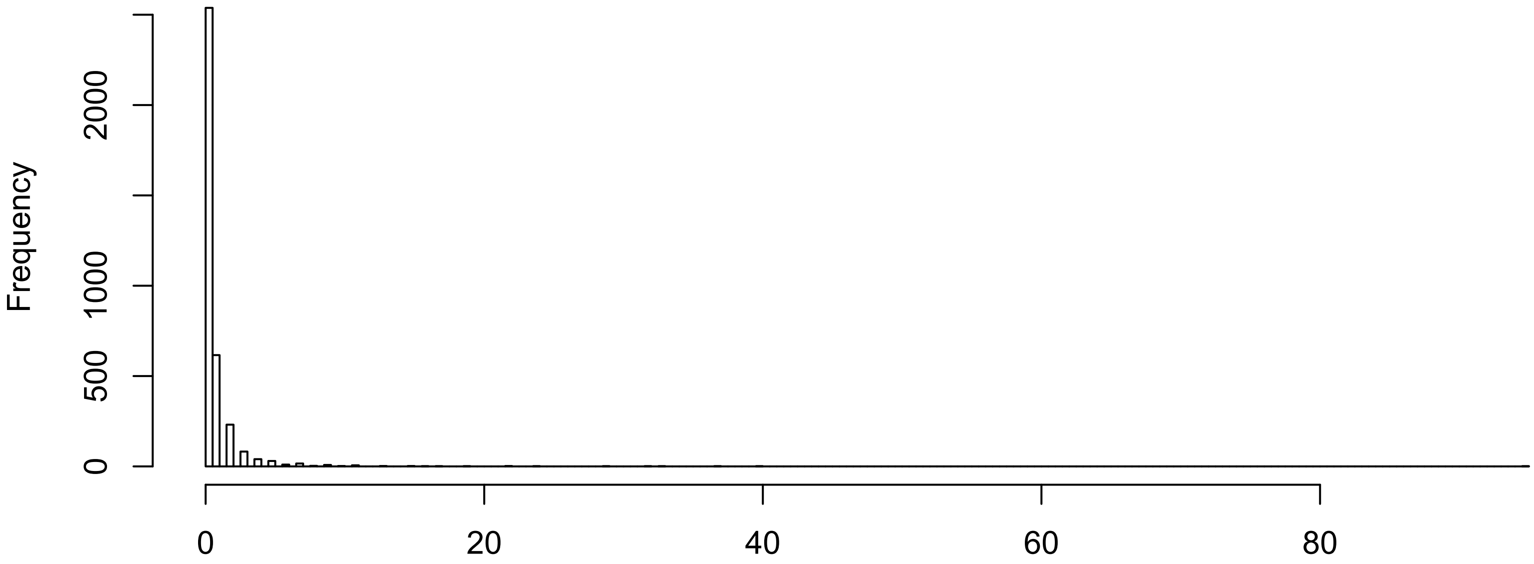
Hunting underdispersion

- Very simple.
  - Just need the mutation counts in each individuals
- Literally no confounding factor(?)
  - All factors push it to the other direction

- But, very weak…need very strong signal
- Start with premature stops (PS) in “essential genes”

Essential genes + PS

VAR=8.84
“expected”=0.9565



PS

Gene SET	DNA Repair	Metabolic	Driver (Vogelstein)	TSG (Vogelstein)
Expected	0.043	0.39	0.0185	0.236
Observed	0.067	1.82	0.0221	0.397

Revised Model

- Trade-off between heterogeneity/power

- Let's stratify the dataset
  - Not underdispersed in Liver HCC and Melanoma

- Caveat: the model needs a little more assumptions now

Next Step

- Rigorous stat test
  - Subsampling to estimate uncertainty in VAR?

- Pinpoint the gene pairs or cliques