

# Lung Precancer Analysis

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# Overview

1 Introduction

2 Materials

3 Methods

4 Results

5 Discussion

6 References

# 1. Introduction

# 1. Introduction

## 1.1. Lung Cancer

# Lung Cancer?

The most common cancer

The most common form of cancer:

12.3 % of all cancers (Minna, Roth, & Gazdar, 2002)

The most important factor

Tobacco

# Cancer Survival Rate in Korea



Figure: Common cancer survival rates (S. Hong et al., 2021)

## Survival rate (More than 5 yr)

- Thyroid: 68.4 %
- Lung: 35.4 %

# Type of Lung Cancer

Types of lung cancer:

- ① Adenocarcinoma (LUAD) (40 %) ★
- ② Squamous cell carcinoma (LUSC) (25 %) ★
- ③ Small cell carcinoma (20 %)
- ④ Large cell carcinoma (10 %)
- ⑤ Adenosquamous carcinoma (< 5 %)
- ⑥ Carcinoid (< 5 %)
- ⑦ Bronchioalveolar (Bronchial gland carcinoma)

(Vincent et al., 1977; Collins, Haines, Perkel, & Enck, 2007)

# 1. Introduction

## 1.2. Non-small cell lung cancer

# Non-small cell lung cancer (NSCLC)

# 1. Introduction

## 1.3. LUAD

# LUAD

# 1. Introduction

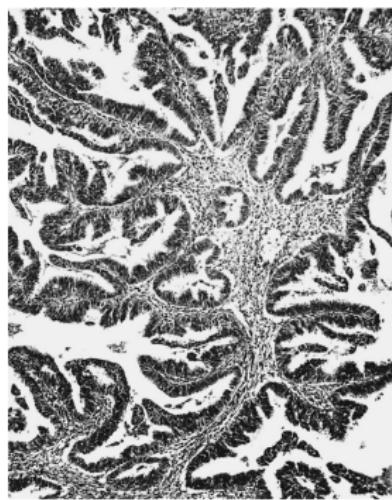
## 1.4. LUSC



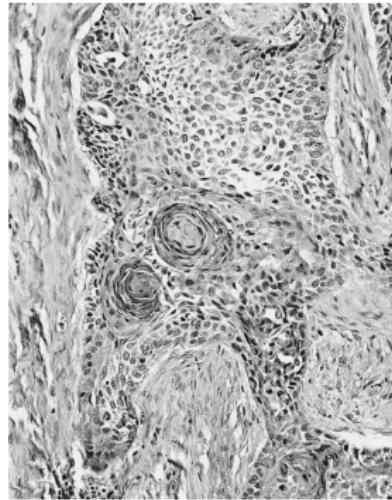
# 1. Introduction

## 1.5. LUAD vs. LUSC

# LUAD vs. LUSC I



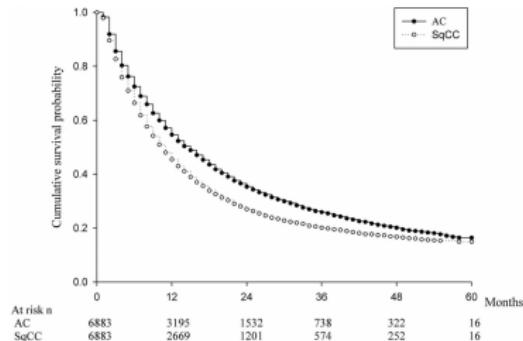
(a) LUAD



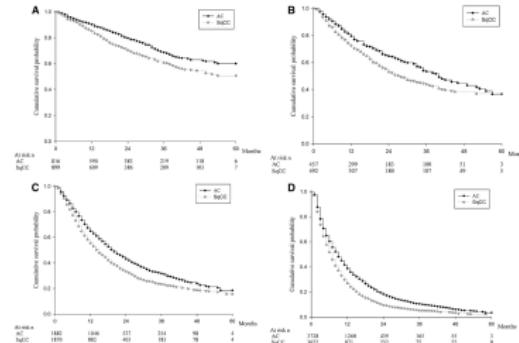
(b) LUSC

**Figure:** LUAD and LUSC histology in Lung cancer (Travis, 2002)

# LUAD vs. LUSC II



(a) All patients



(b) By cancer stages

**Figure:** Kaplan-Meiere survival curves for LUAD & LUSC (B.-Y. Wang et al., 2020)

## Findings

LUSC is more dangerous than LUAD.  $\therefore p < 0.001$

# 1. Introduction

## 1.6. Study Objectives

# Study Objectives

## Find different mutations

- between WES vs. WTS
- from cancer vs. precancer

## Pathway examine

- with the mutation of WES & RNA-seq
- with immune-depleted animal models

## Ultra-deep sequencing

to find an *infinitesimal* quantity of Non-Circulating Tumor DNA

- from blood
- from urine
- from bronchus

## 2. Materials

# Lung Cancer Data

- Exome (WES) (n=289) + Transcriptome (WTS) (n=166)
- Normal + {Primary, CIS + AIS, AAH, Dysplasia, MIA}
  - Carcinoma in situ
  - Adenocarcinoma in situ
  - Atypical adenomatous hyperplasia
  - Dysplasia
  - Minimally invasive adenocarcinoma
- Adenocarcinoma (LUAD) & Squamous cell carcinoma (LUSC)
  - ① Normal → AAH → AIS → MIA → LUAD (n=28)
  - ② Normal → Dysplasia → CIS → LUSC (n=80)

## 2. Materials

### 2.1. WES Data

# WES Data Composition

Table: Number of WES samples

Cancer Subtype	Stage	Number of Samples
LUSC	Normal	77
	Dysplasia	5
	AAH	8
	CIS+AIS	73
	Primary	77
	Total	240
LUAD	Normal	18
	AAH	15
	CIS+AIS	9
	MIA	1
	Primary	18
	Total	61

# WES Data Composition with Recurrence I

Table: LUSC WES Data with Recurrence

Recurrence?	Stage	Number of Samples	
		Normal	Dysplasia
Recurrence	Normal	14	
	Dysplasia		4
	CIS+AIS	12	
	Primary	14	
	Total	44	
Non-recurrence	Normal	63	
	Dysplasia		1
	AAH	8	
	CIS+AIS	61	
	Primary	63	
	Total	196	

# WES Data Composition with Recurrence II

Table: LUAD WES Data with Recurrence

Recurrence?	Stage	Number of Samples	
		Normal	AAH
Recurrence	Normal	5	8
	AAH	2	5
	CIS+AIS	2	5
	Primary	5	20
	Total	20	
Non-recurrence	Normal	13	7
	AAH	7	1
	CIS+AIS	7	13
	MIA	1	41
	Primary	13	
	Total	41	

# WES Data Composition with Smoking I

Table: LUSC WES Data with Smoking

Smoking?	Stage	Number of Samples	
		Normal	Total
Never	Normal	3	
	CIS+AIS	3	
	Primary	3	
	Total	9	
Ex	Normal	41	
	Dysplasia	1	
	AAH	4	
	CIS+AIS	40	
	Primary	41	
	Total	127	
Current	Normal	33	
	Dysplasia	4	
	AAH	4	
	CIS+AIS	30	
	Primary	33	
	Total	104	

# WES Data Composition with Smoking II

Table: LUAD WES Data with Smoking

Smoking?	Stage	Number of Samples	
		Normal	Total
Never	Normal	1	
	CIS+AIS	1	
	Primary	1	
	Total	3	
Ex	Normal	10	
	AAH	9	
	CIS+AIS	6	
	Primary	10	
	Total	35	
Current	Normal	7	
	AAH	6	
	CIS+AIS	2	
	MIA	1	
	Primary	7	
	Total	23	

## 2. Materials

### 2.2. WTS Data

# WTS Data Composition

Table: Number of WTS samples

Cancer Subtype	Stage	Number of Samples	
		Normal	Dysplasia
LUSC	Normal	17	
	Dysplasia		2
	CIS+AIS	34	
	Primary	36	
	Total	89	
LUAD	Normal	13	
	AAH		1
	CIS+AIS	5	
	Primary	6	
	Total	25	

# WTS Data Composition with Recurrence I

Table: LUSC WTS Data with Recurrence

Recurrence?	Stage	Number of Samples	
		Normal	Dysplasia
Recurrence	Normal	1	
	Dysplasia		1
	CIS+AIS		5
	Primary		6
	Total	13	
Non-recurrence	Normal	16	
	Dysplasia		1
	CIS+AIS		29
	Primary		30
	Total	76	

# WTS Data Composition with Recurrence II

Table: LUAD WTS Data with Recurrence

Recurrence?	Stage	Number of Samples	
		Normal	CIS+AIS
Recurrence	Normal	2	
	CIS+AIS		1
	Primary		1
	Total	4	
Non-recurrence	Normal	11	
	AAH		1
	CIS+AIS		4
	Primary		5
	Total	21	

# WTS Data Composition with Smoking I

Table: LUSC WTS Data with Smoking

Smoking?	Stage	Number of Samples	
		Normal	AIS
Never	Normal	1	
	CIS+AIS	1	
	Primary	2	
	Total	4	
Ex	Normal	8	
	Dysplasia	1	
	CIS+AIS	21	
	Primary	22	
	Total	52	
Current	Normal	8	
	Dysplasia	1	
	CIS+AIS	12	
	Primary	12	
	Total	33	

# WTS Data Composition with Smoking II

Table: LUAD WTS Data with Smoking

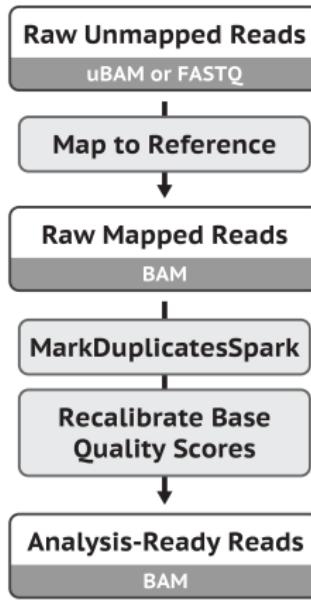
Smoking?	Stage	Number of Samples	
Never	Normal	10	
	AAH	1	
	CIS+AIS	3	
	Primary	4	
	Total	18	
Ex	Normal	3	
	CIS+AIS	1	
	Primary	1	
	Total	5	
Current	CIS+AIS	1	
	Primary	1	
	Total	2	

### 3. Methods

### 3. Methods

#### 3.1. Workflows

# Data pre-processing for variant discovery



**Figure:** Data pre-processing for variant discovery (Van der Auwera et al., 2013; DePristo et al., 2011)

# Somatic short variant discovery

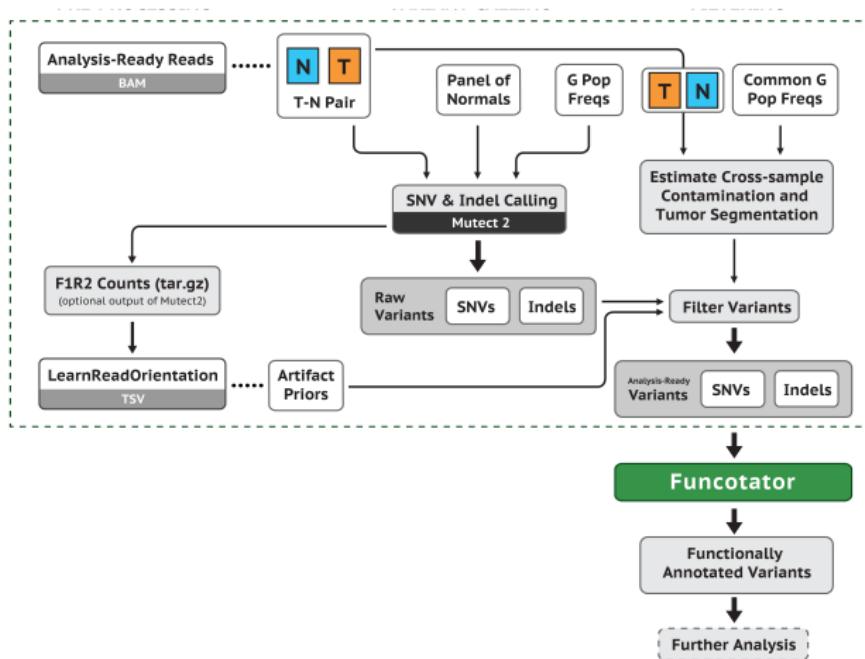
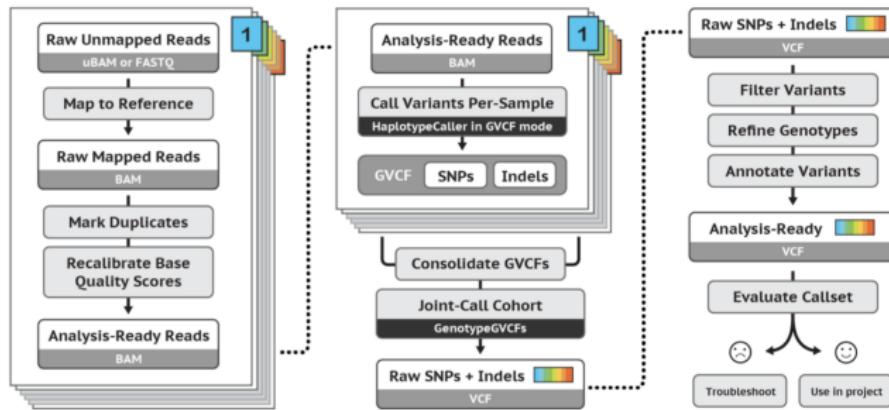


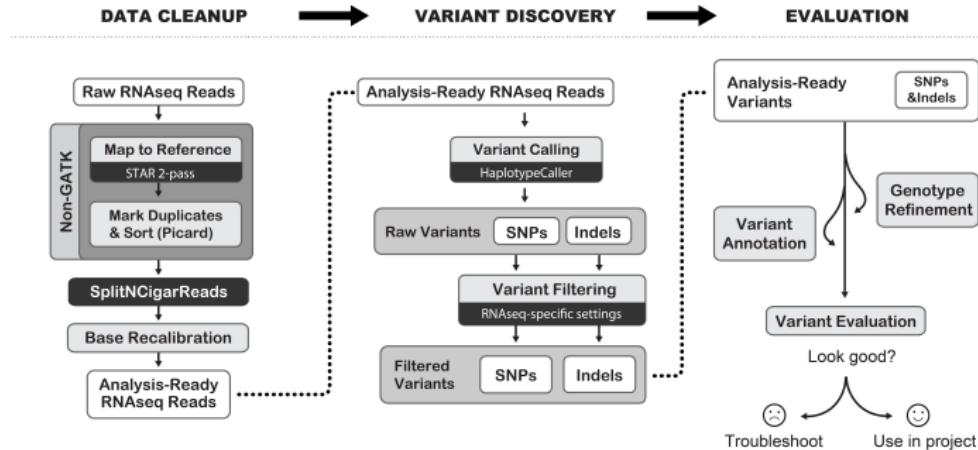
Figure: Somatic short variant (SNVs + Indels) discovery workflow (Van der Auwera et al., 2013; DePristo et al., 2011)

# Germline short variant discovery



**Figure:** Germline short variant (SNVs + Indels) discovery workflow (Van der Auwera et al., 2013; DePristo et al., 2011)

# RNA-seq short variant discovery



**Figure:** RNA-seq short variant (SNVs + Indels) discovery workflow (Van der Auwera et al., 2013; DePristo et al., 2011)

## 4. Results

## 4. Results

### 4.1. Quality Checks with FastQC

# FastQC?

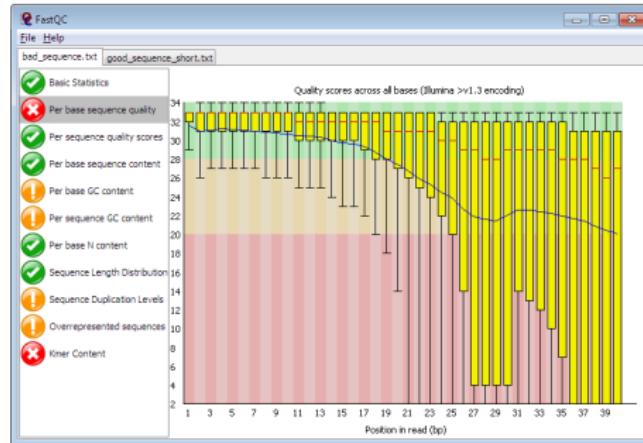


Figure: Example of FastQC Result (Andrews et al., 2012)

- A quality check tool for sequence data
- Give an overview that which test may be problems

# FastQC on WES

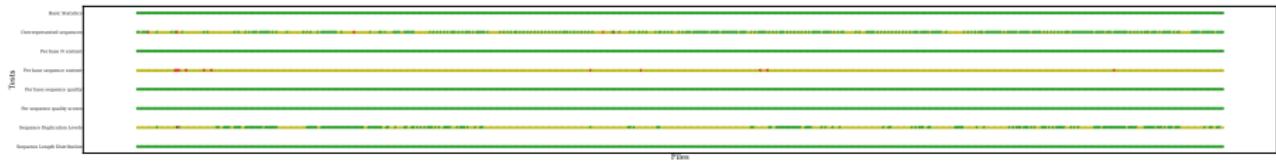
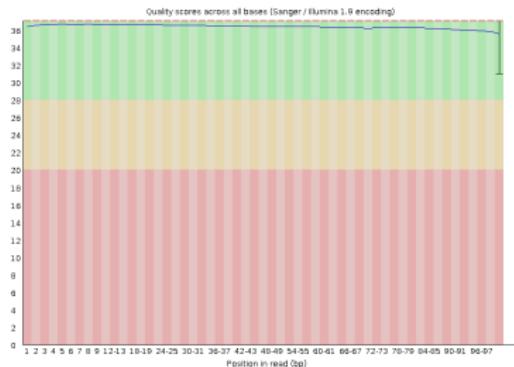


Figure: FastQC with WES data

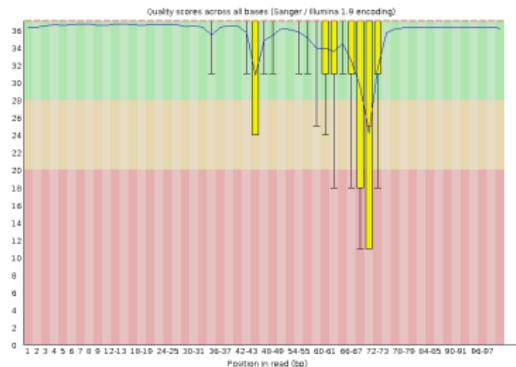
Failure on 33P1 sample

33P1 is excluded at further analysis.

# Failure on 33P1 I



(a) 33N



(b) 33P1

Figure: Per Base Sequence Quality Results

# Failure on 33P1 II

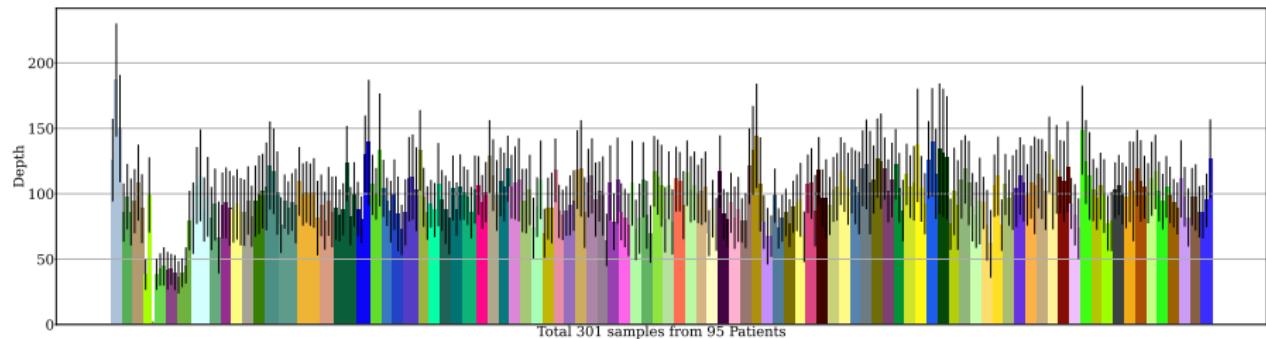


Figure: Coverage Depth Plot

# FastQC on WTS

Tests

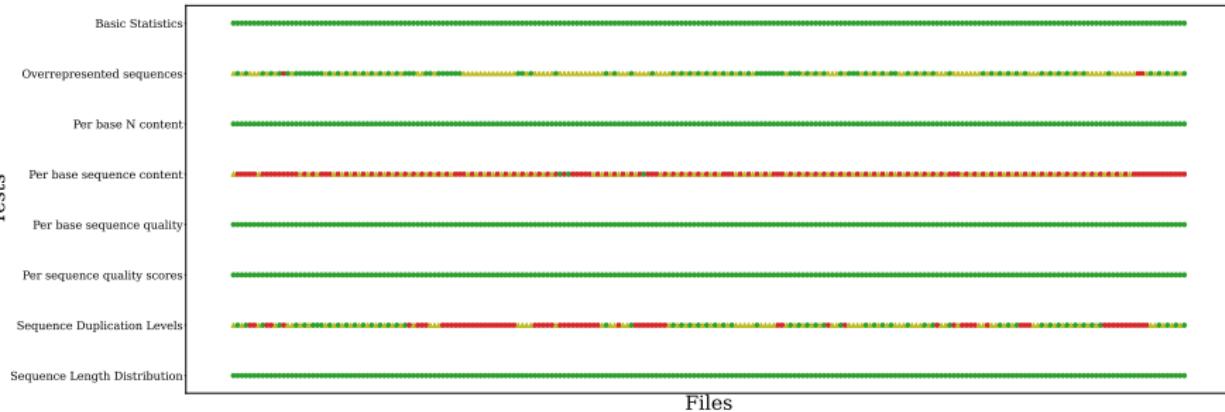


Figure: FastQC with WTS data

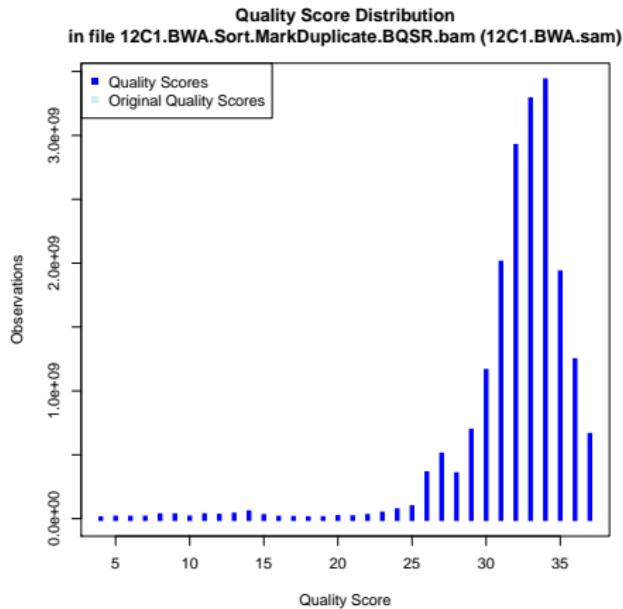
All sample are good to analysis

∴ No sample has more than 5 failures.

## 4. Results

### 4.2. Quality Checks with Picard

# Picard?



**Figure:** Quality Distribution of 12C1 sample

# Quality Distribution Plot

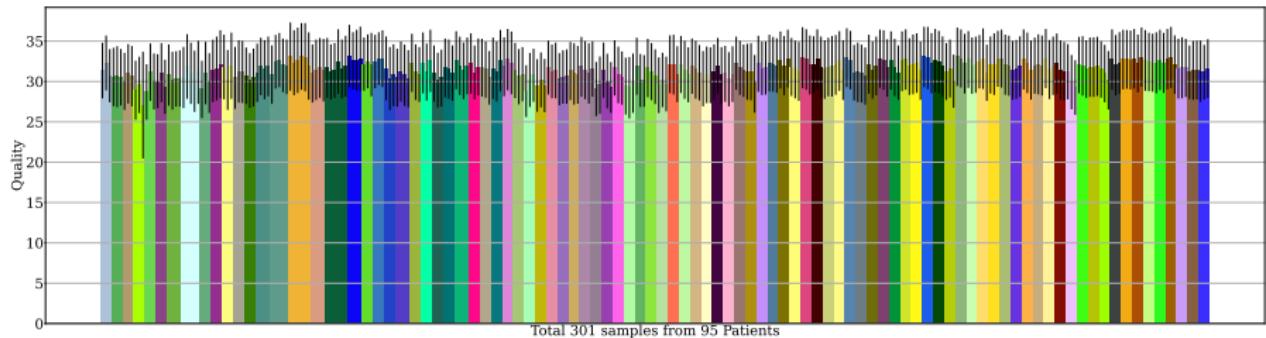


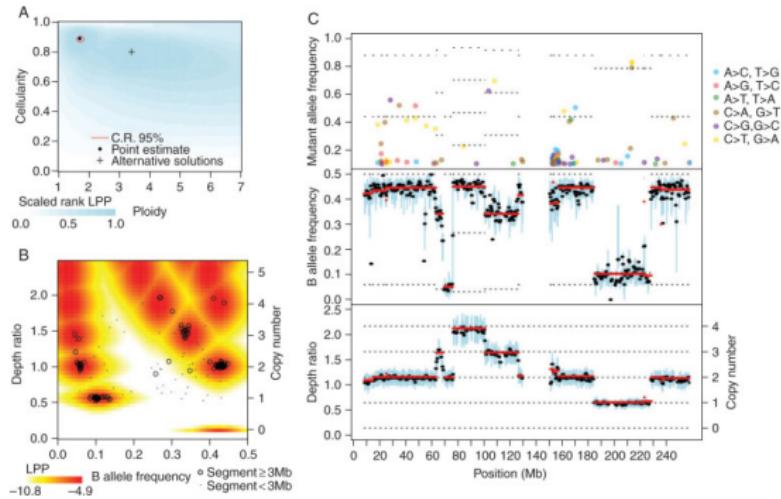
Figure: Quality Distribution by Samples

# Findings in Picard

## 4. Results

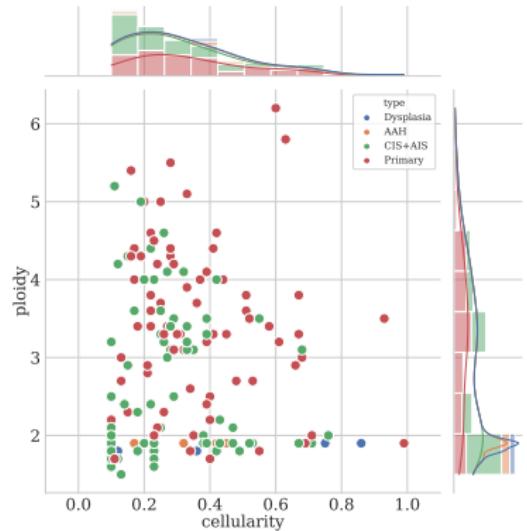
### 4.3. Copy Number Variations

# Sequenza?

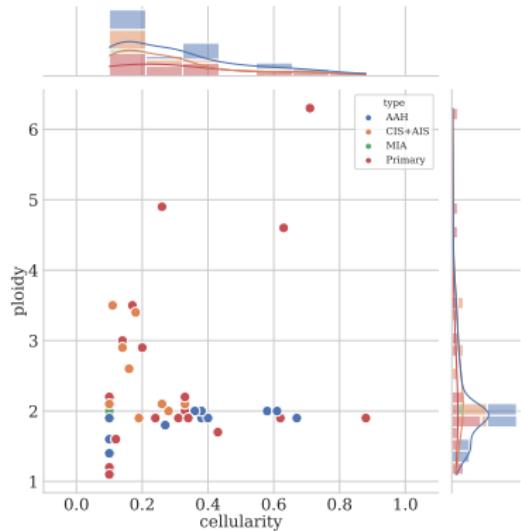


**Figure:** Representative Output of the Sequenza (Favero et al., 2015)

# Cellularity & Ploidy on WES



(a) LUSC Samples



(b) LUAD Samples

Figure: Cellularity and Ploidy from Sequenza

# LUSC in CNV Plot I

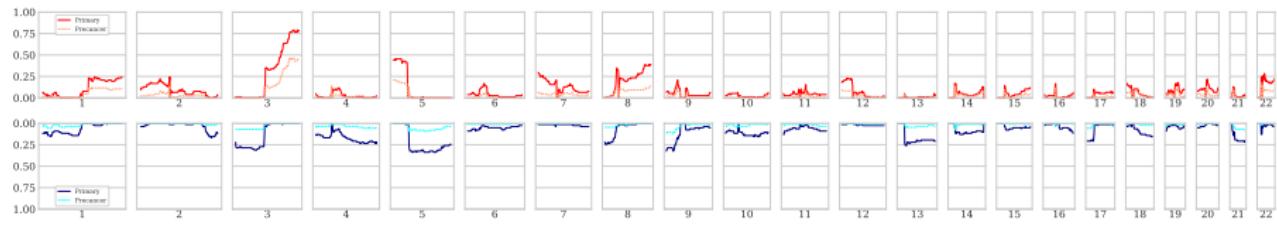


Figure: LUSC in CNV Plot

# LUSC in CNV Plot II

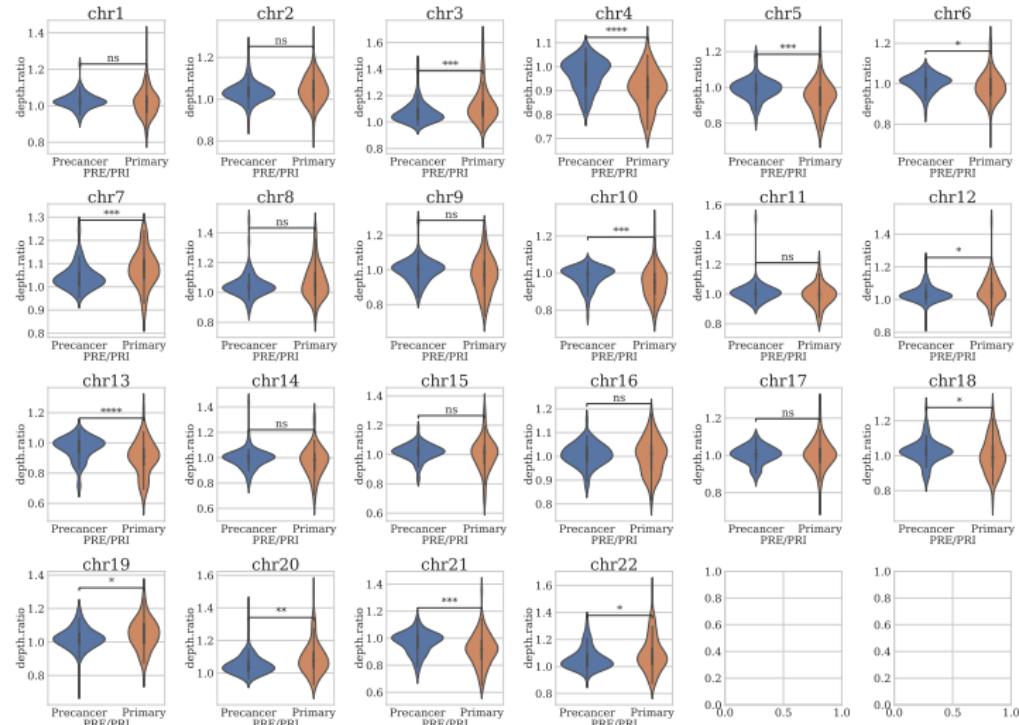


Figure: CNV Violin Plot in LUSC

# LUSC with Recurrence in CNV Plot I

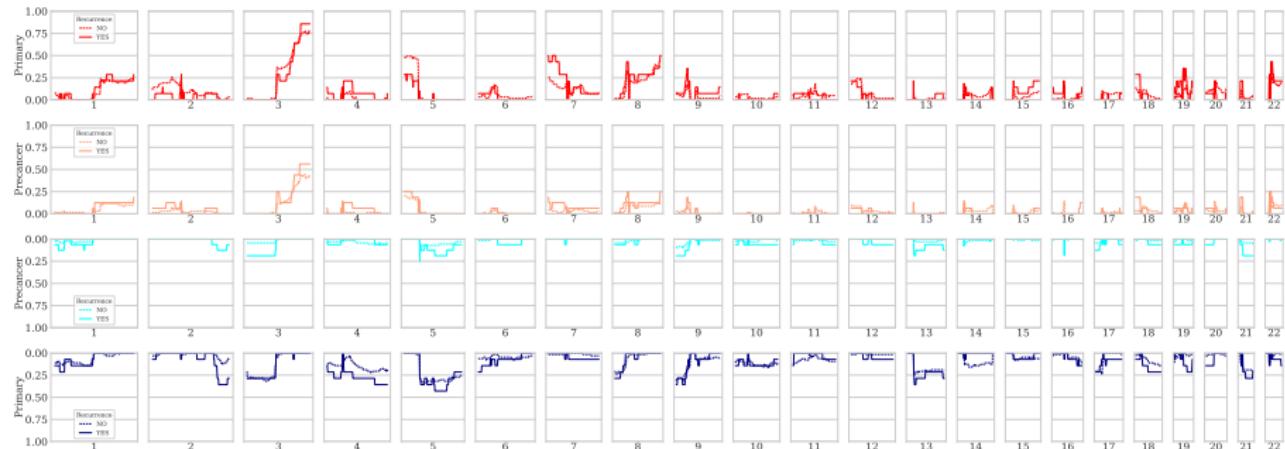


Figure: LUSC with Recurrence in CNV Plot

# LUSC with Recurrence in CNV Plot II

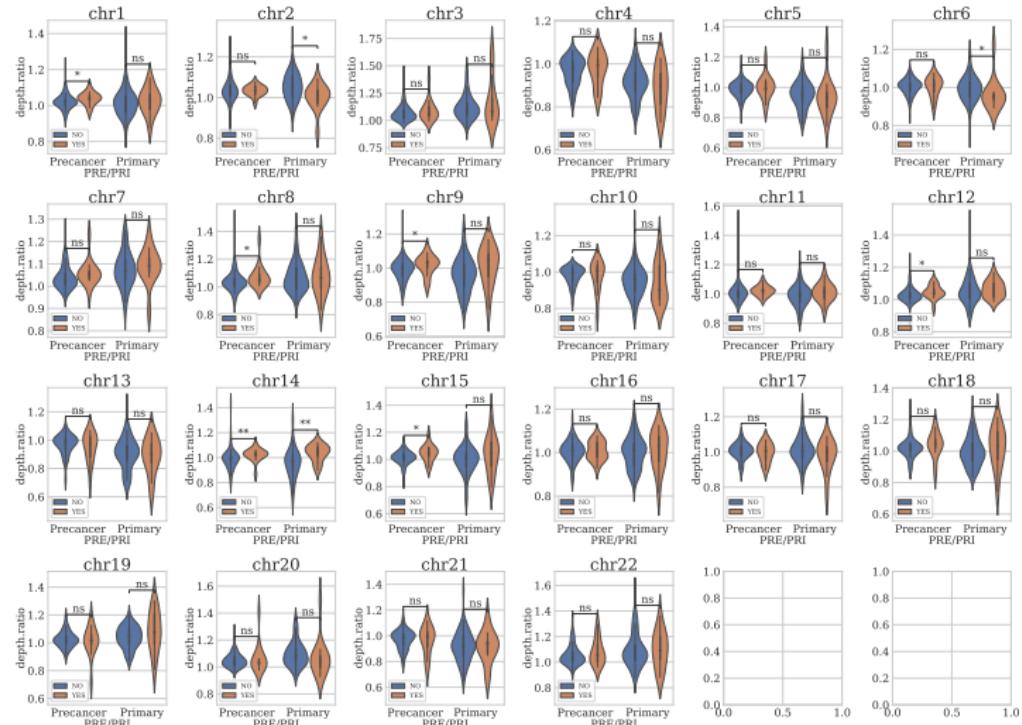


Figure: CNV Violin Plot in LUSC with Recurrence

# LUSC with Smoking in CNV Plot

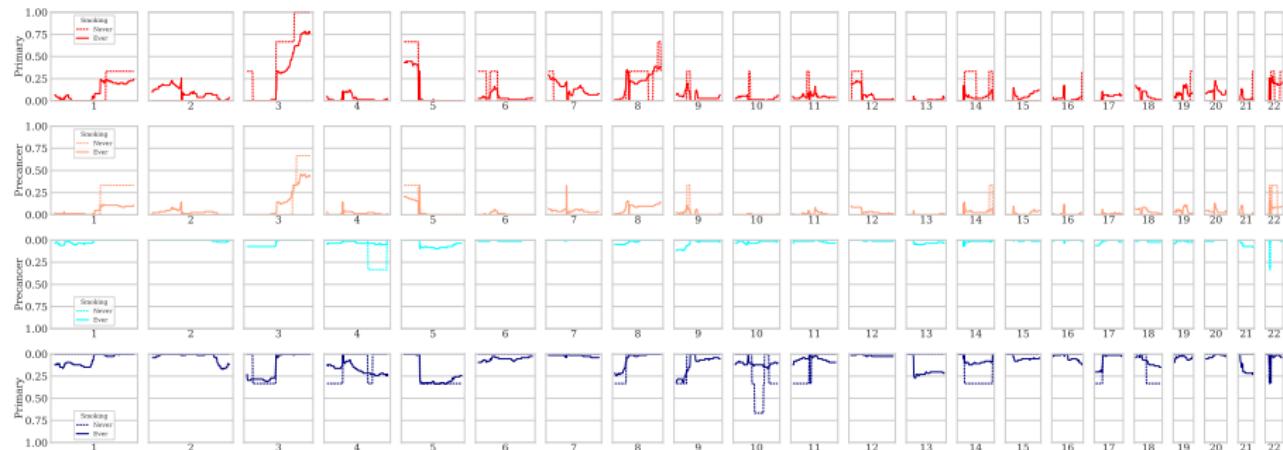


Figure: LUSC with Smoking in CNV Plot

# LUSC with Smoking in CNV Plot II

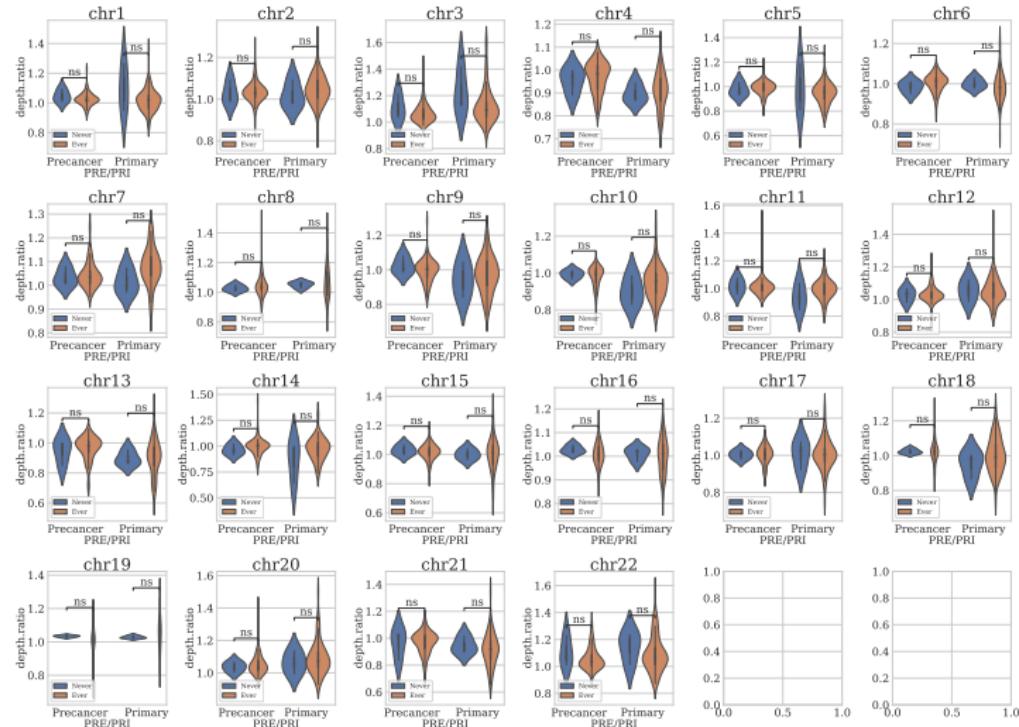


Figure: CNV Violin Plot in LUSC with Smoking

# LUAD in CNV Plot I

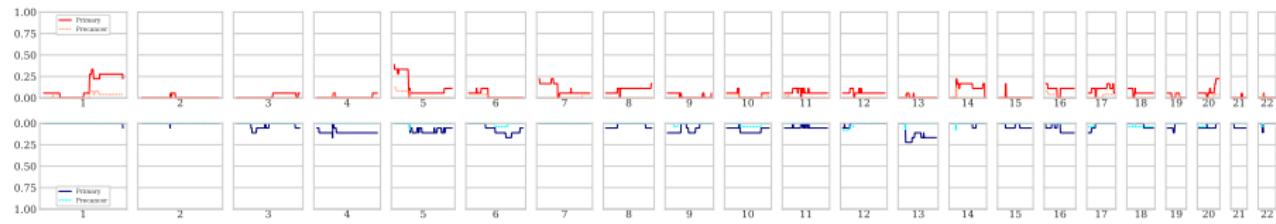


Figure: LUAD in CNV Plot

# LUAD in CNV Plot II

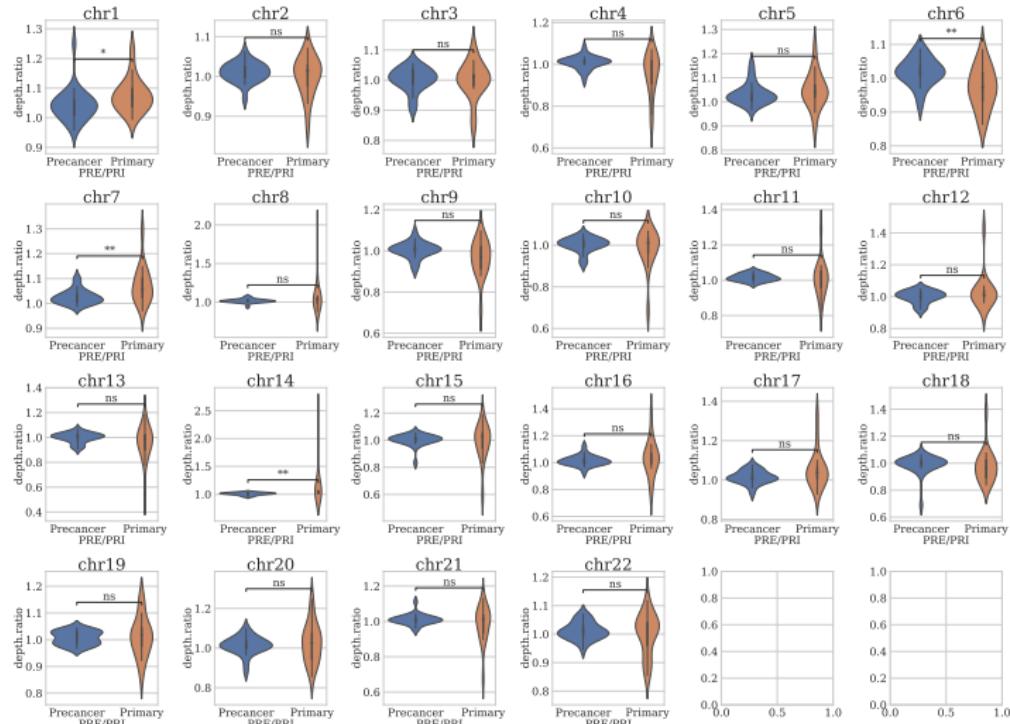


Figure: CNV Violin Plot in LUAD

# LUAD with Recurrence in CNV Plot I

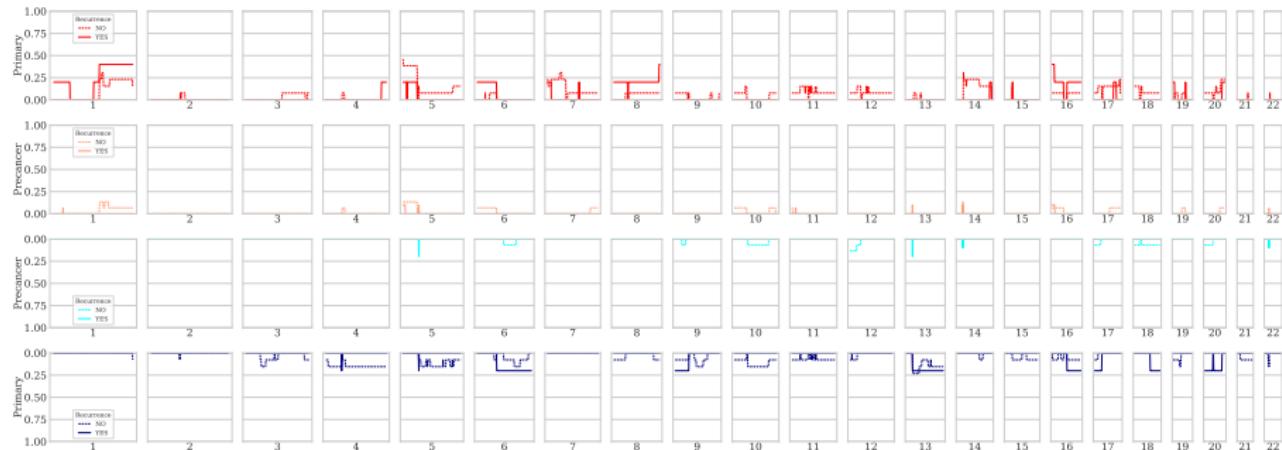


Figure: LUAD with Recurrence in CNV Plot

# LUAD with Recurrence in CNV Plot II

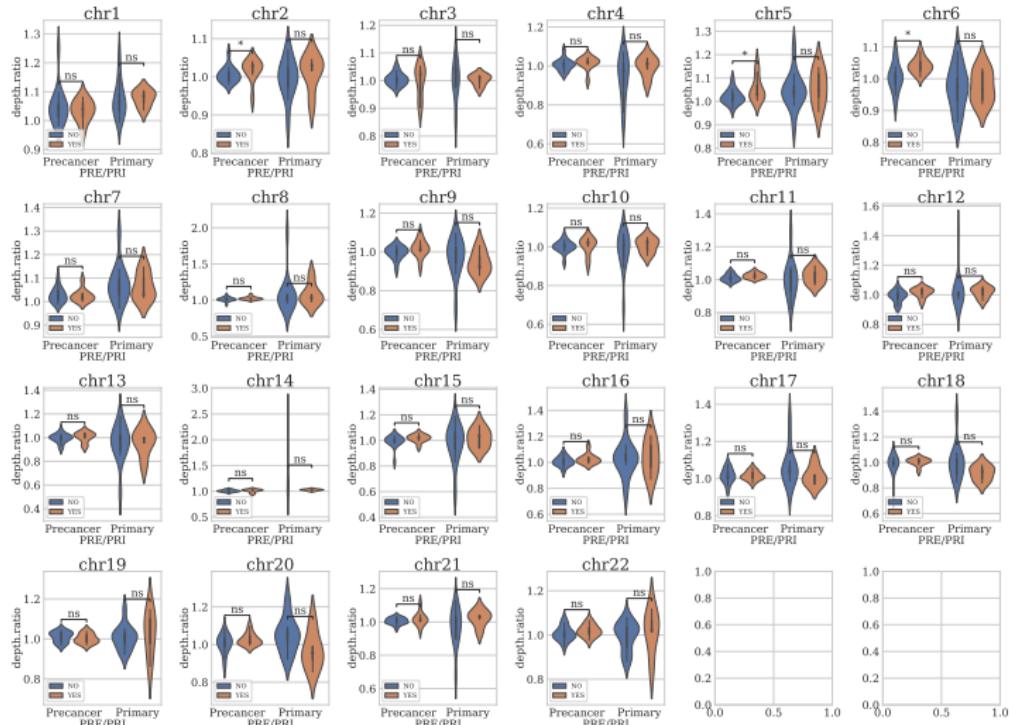


Figure: CNV Violin Plot in LUAD with Recurrence

# LUAD with Smoking in CNV Plot I

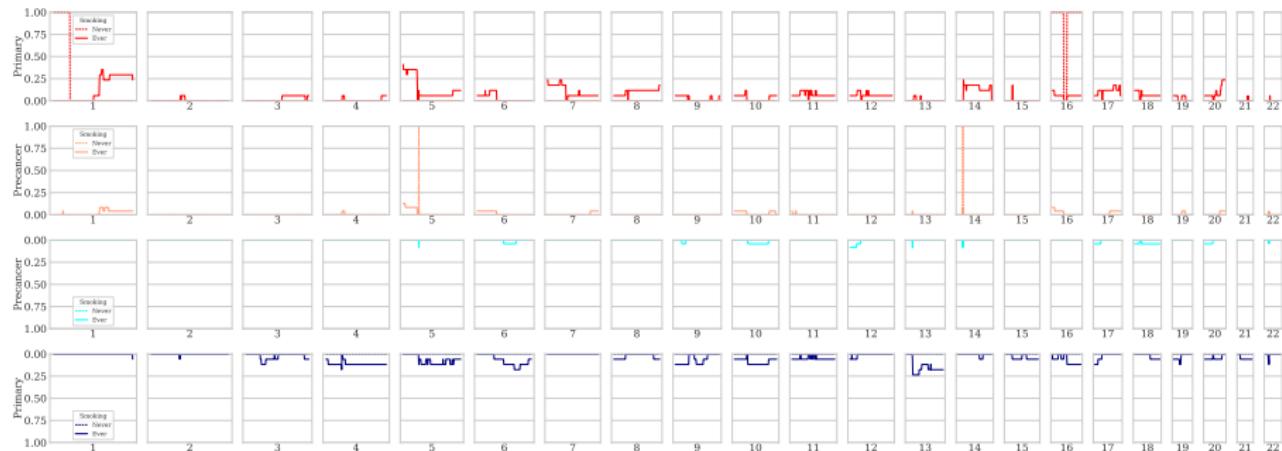


Figure: LUAD with Smoking in CNV Plot

# LUAD with Smoking in CNV Plot II

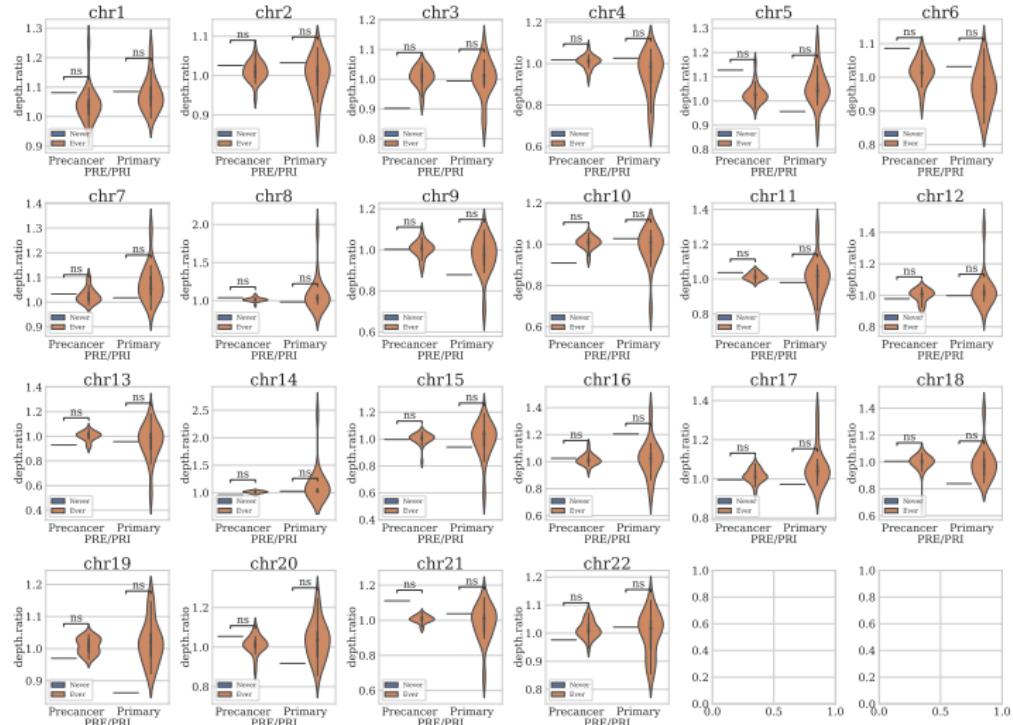


Figure: CNV Violin Plot in LUAD with Smoking

# Findings in Sequenza

# PureCN?

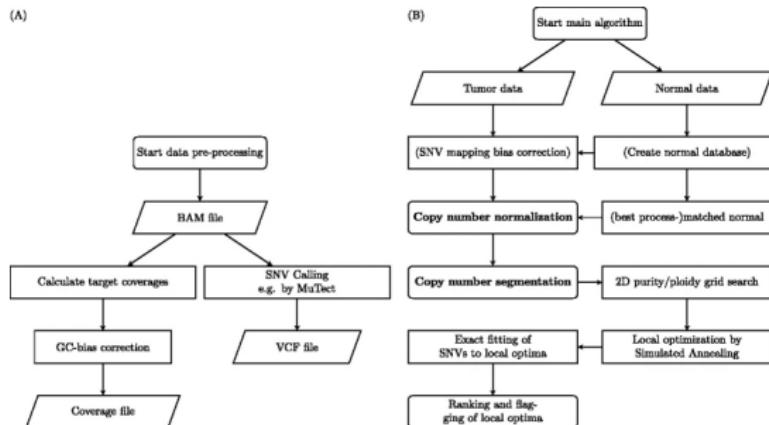
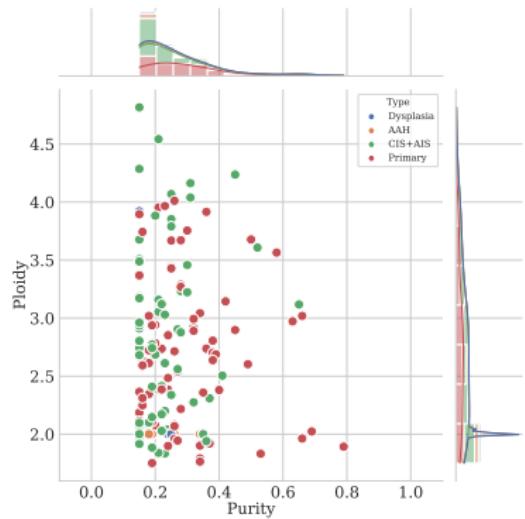
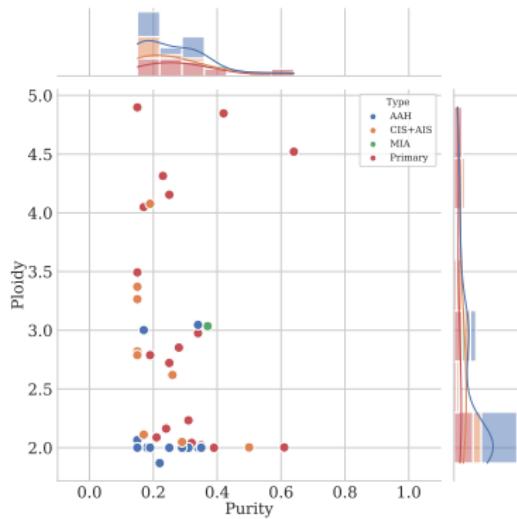


Figure: Flowchart of the PureCN data pre-processing pipeline (Riester et al., 2016)

# Purity & Ploidy on WES



(a) LUSC Samples



(b) LUAD Samples

Figure: Cellularity and Ploidy from PureCN

# LUSC in CNV Plot I

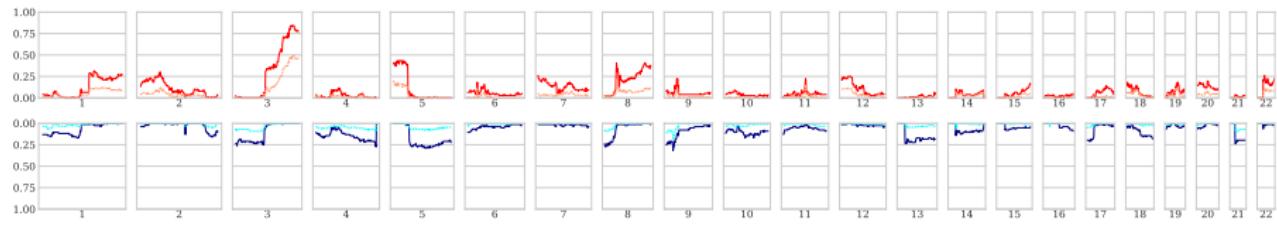


Figure: LUSC in CNV Plot

# LUSC with Recurrence in CNV Plot I

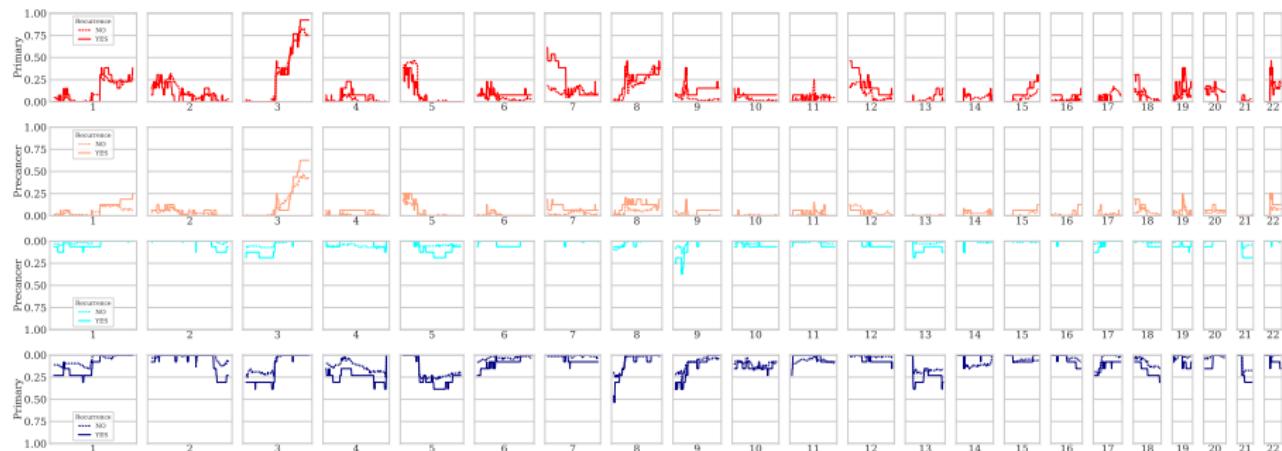


Figure: LUSC with Recurrence in CNV Plot

# LUSC with Smoking in CNV Plot I

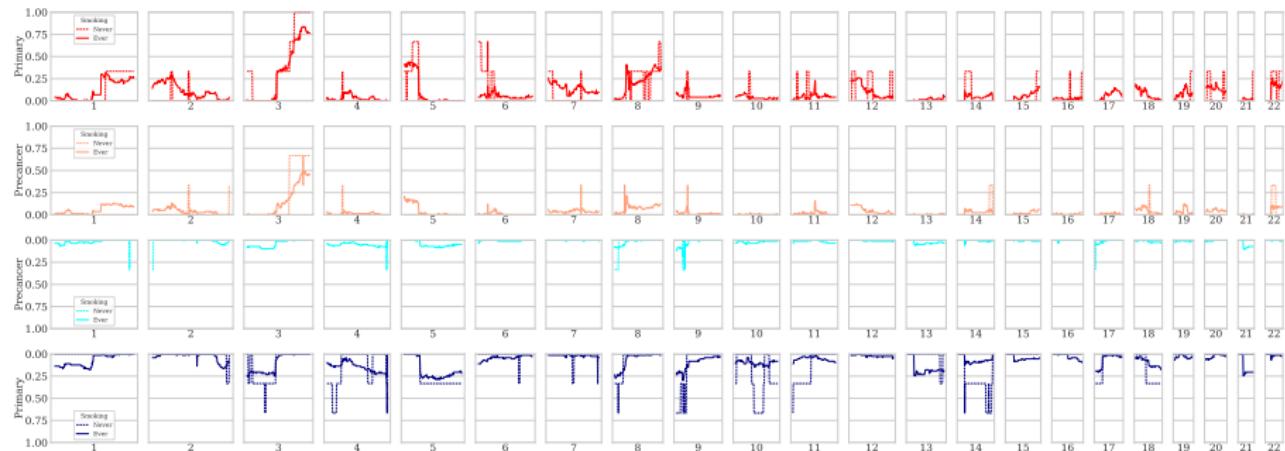


Figure: LUSC with Smoking in CNV Plot

# LUAD in CNV Plot I

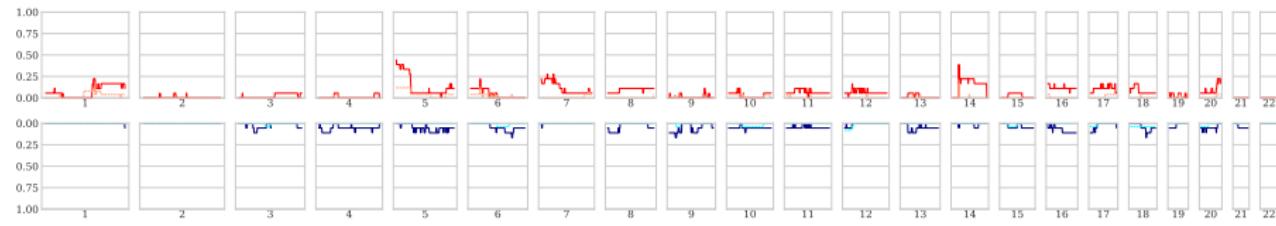


Figure: LUAD in CNV Plot

# LUAD with Recurrence in CNV Plot I

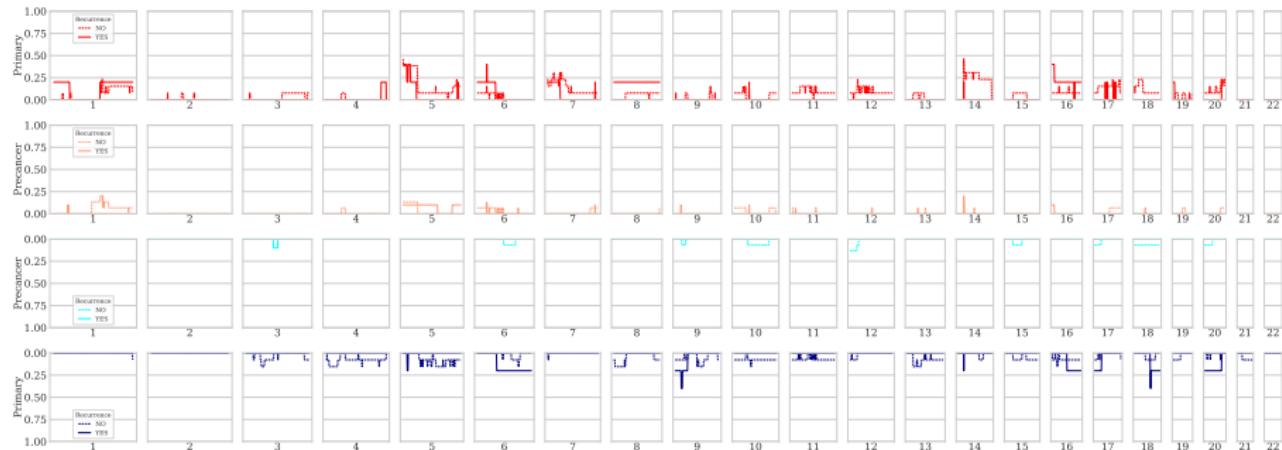


Figure: LUAD with Recurrence in CNV Plot

# LUAD with Smoking in CNV Plot I

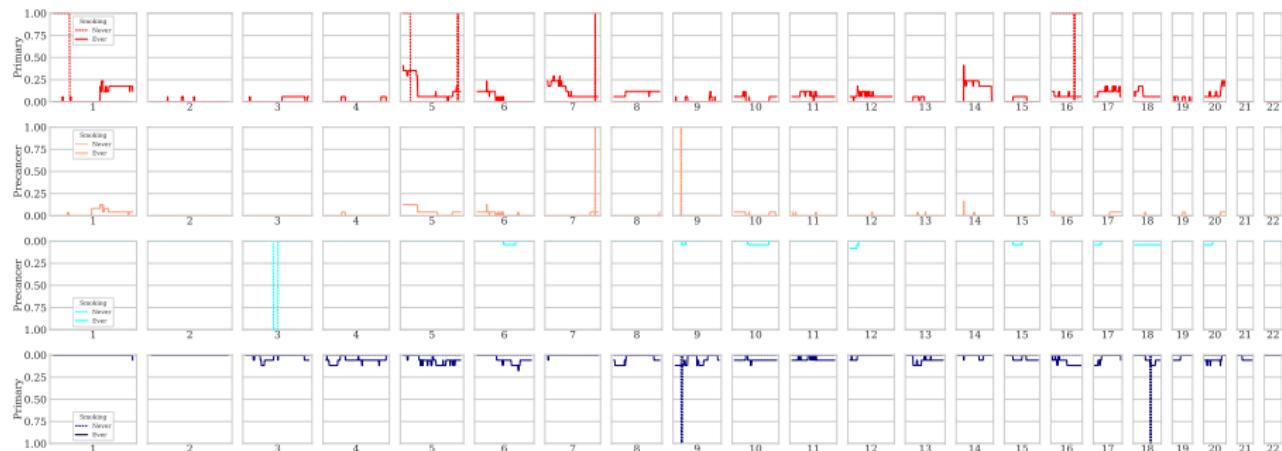


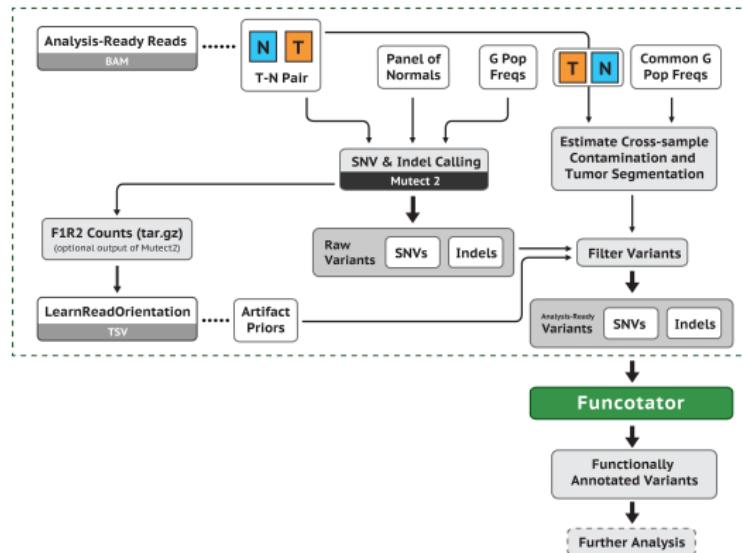
Figure: LUAD with Smoking in CNV Plot

# Findings in PureCN

## 4. Results

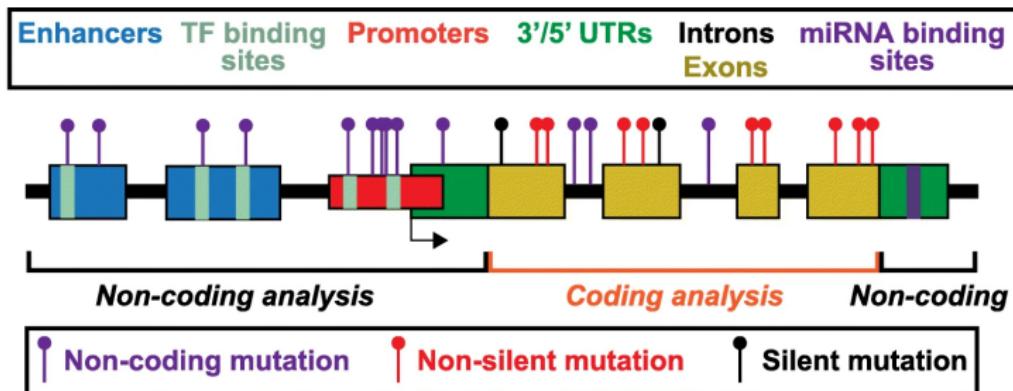
### 4.4. SNVs Analysis

# Mutect2?



**Figure:** Somatic short variant discovery workflow (Van der Auwera et al., 2013; DePristo et al., 2011)

# MutEnricher?



## Analysis summary:

### Inputs:

- Somatic mutations
- Features of interest:
  - Coding genes
  - Non-coding regions
- Genomic covariates (optional)

### Analyses:

- Background calculations:
  - global, local, or covariate clustered
- Mutation enrichments:
  - coding/non-coding modules

### Outputs:

- Gene or non-coding region enrichments:
  - Overall genes/regions
  - Hotspots
  - Combined

**Figure:** Schematic representation of MunEnricher's analysis procedures (Soltis et al., 2020)

# CoMut?

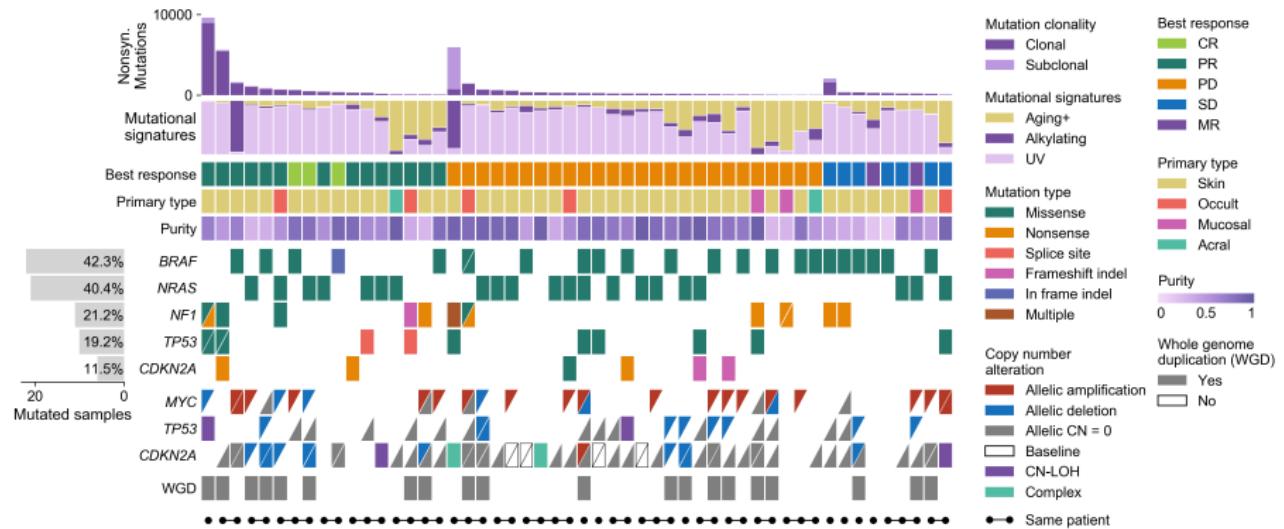


Figure: A comutation plot generated with CoMut (Crowdis et al., 2020)

# Driver Gene Selection Strategy

COSMIC Cancer Gene Census (Tate John et al., 2018)

Gene  $\in$  CGC Tier 1 set

Fisher FDR

Fisher FDR  $< 0.05$

Fisher P-value

Fisher P-value  $< 0.05$

Gene P-value

Gene P-value  $< 0.05$

# Somatic Variant in LUSC

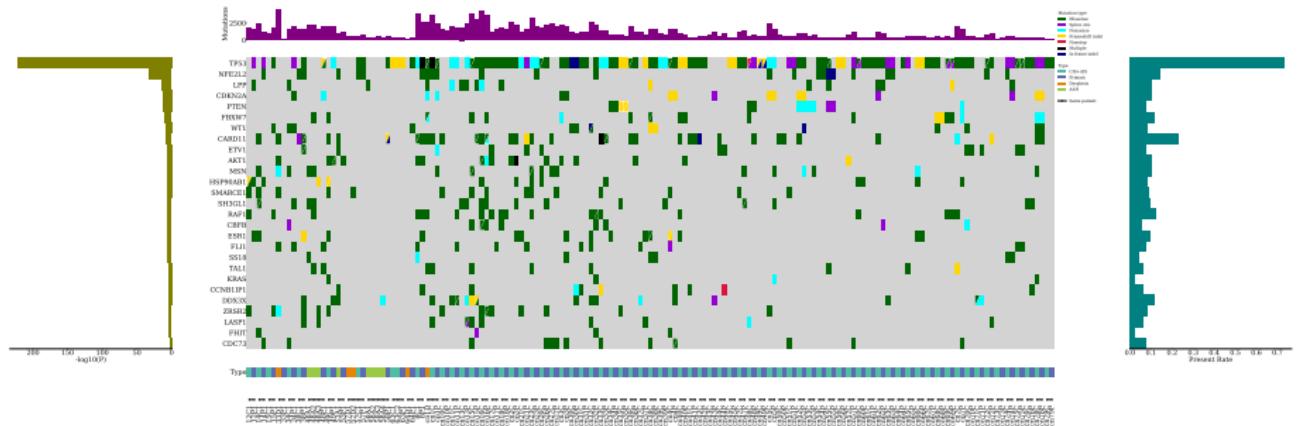


Figure: CoMut Plot with LUSC Patients

# Somatic Variant in LUSC with Recurrence

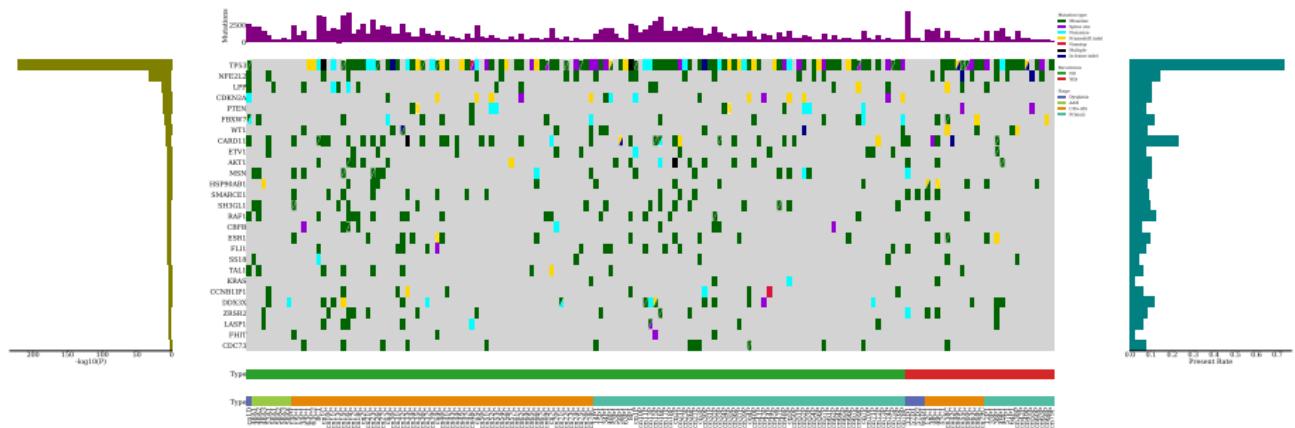


Figure: CoMut Plot in LUSC Patients with Recurrence

# Somatic Variant in LUAD

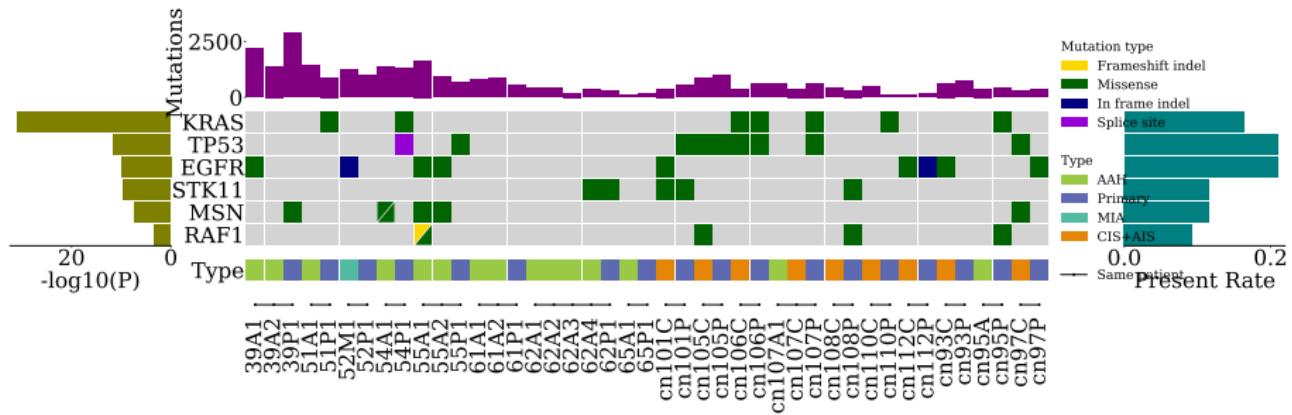


Figure: CoMut Plot with LUAD Patients

# Somatic Variant in LUAD with Recurrence

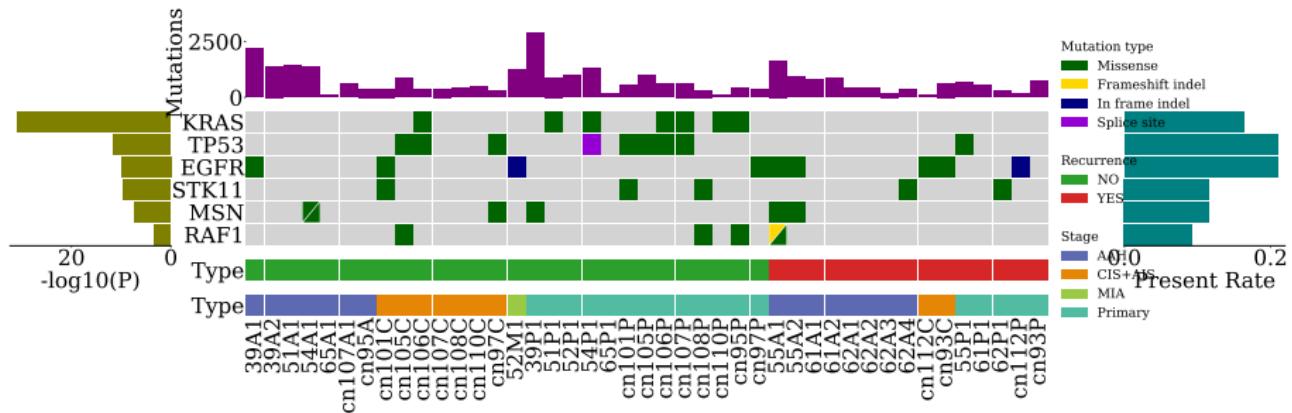


Figure: CoMut Plot in LUAD Patients with Recurrence

# Findings in SNVs Analysis

## 4. Results

### 4.5. VAF Analysis

# VAF?

- Variant allele frequency
- VAF = Alternative allele read count/Total read count
- To find tumor evolution

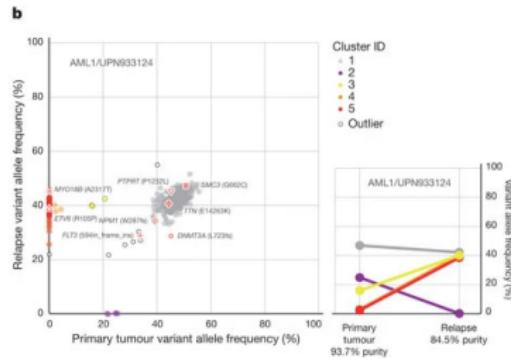
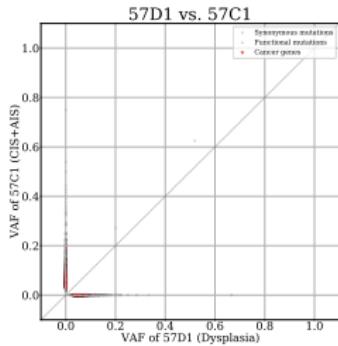
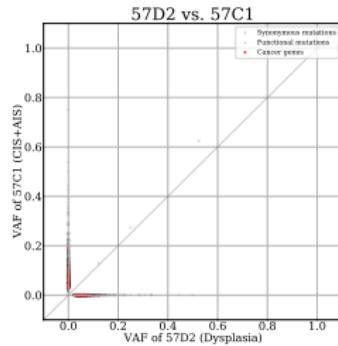


Figure: VAF distribution of validated mutations (Ding et al., 2012)

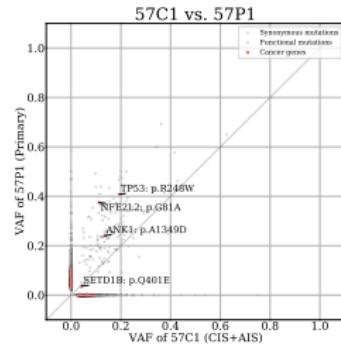
# VAF Plots I



(a) Dysplasia + CIS



(b) Dysplasia + CIS



(c) CIS + Primary

Figure: VAF plots in patient #57

# PyClone?

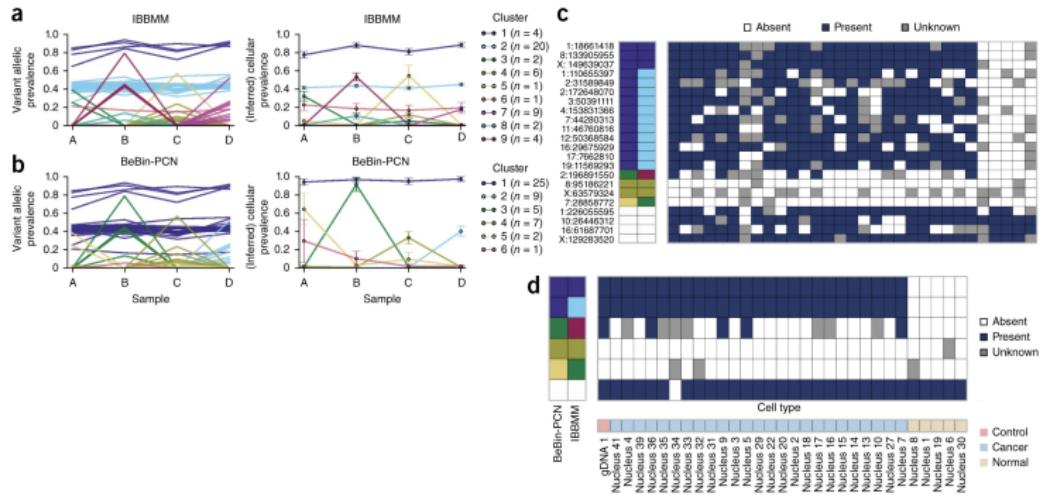


Figure: Analysis of multiple samples by PyClone (Roth et al., 2014)

# PyClone Plots I

# Findings in VAF Analysis

## 4. Results

### 4.6. Tumor Evolution Trajectories Analysis

# Revolver?

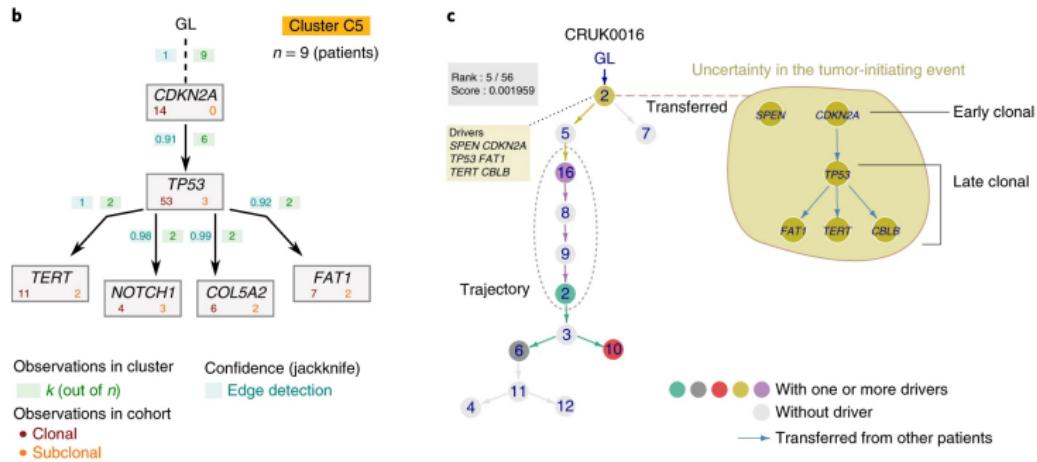


Figure: Repeated Evolutionary Trajectories (Caravagna et al., 2018)

## Revolver in LUSC I

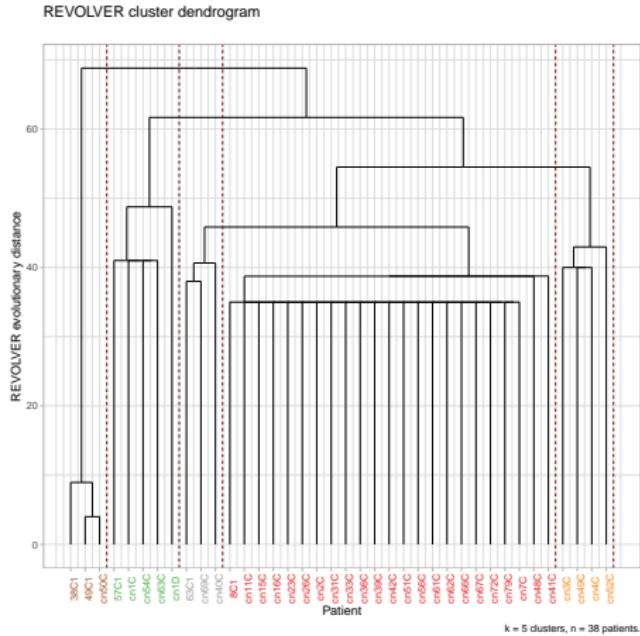


Figure: Dendrogram analysis in LUSC

# Revolver in LUSC II

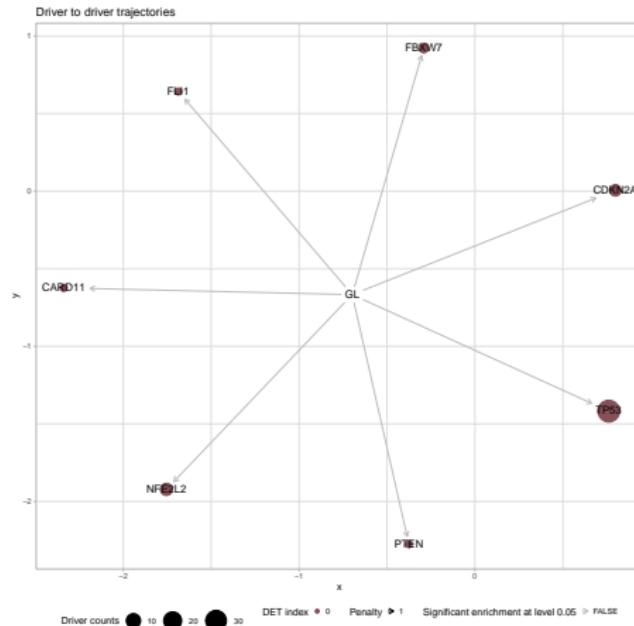


Figure: Driver analysis in LUSC

# Revolver in LUAD I

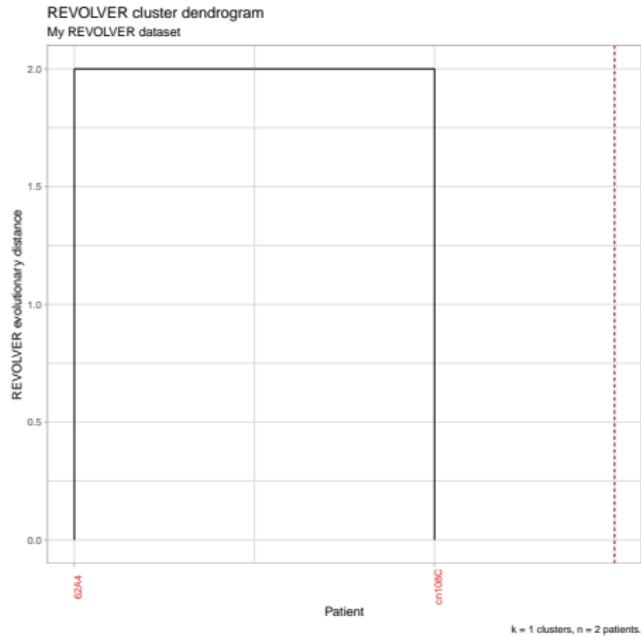


Figure: Dendrogram analysis in LUAD

# Revolver in LUAD II

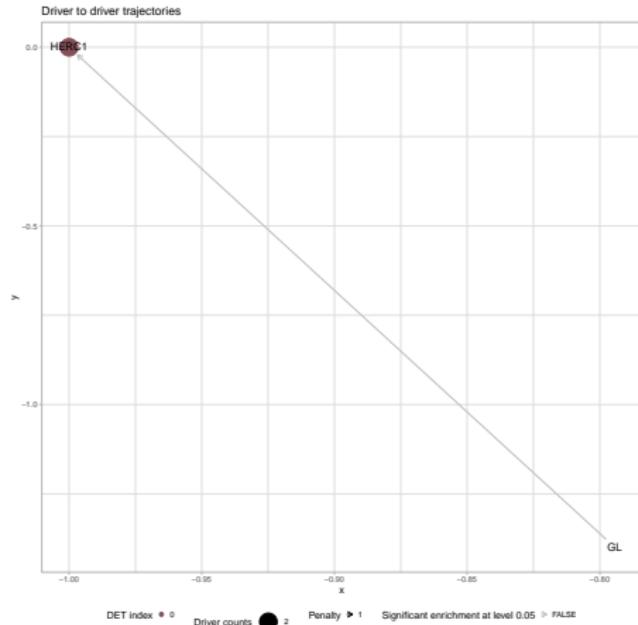


Figure: Driver analysis in LUAD

# Findings in Tumor Evolution Trajectories Analysis

## 4. Results

### 4.7. Differences in Gene Expression Levels

# RSEM?

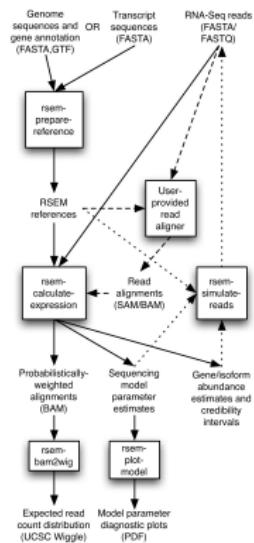


Figure: RSEM workflow (Li & Dewey, 2011)

# DESeq2?

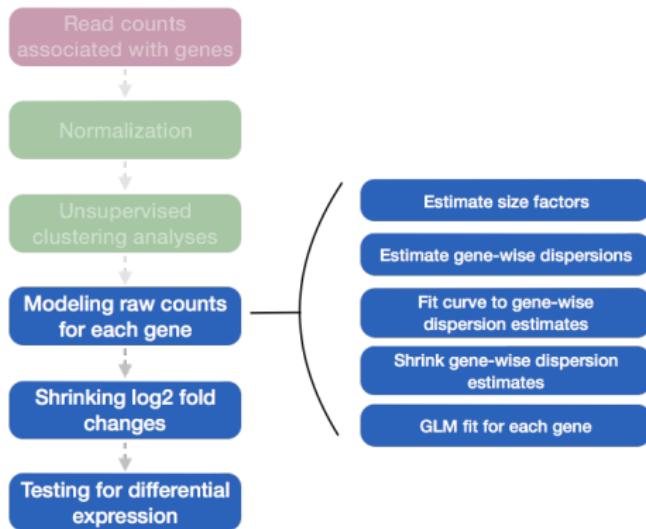


Figure: DESeq2 workflow (Love, Huber, & Anders, 2014)

# DEG Selection Strategy

DEG: differentially expressed genes

## Fold Change

$$\log_2(\text{Fold Change}) > 1 \vee \log_2(\text{Fold Change}) < -1$$

## P-value

$$P\text{-value} < 0.05$$

## Adjusted P-value

$$P_{adj} < 0.05$$

# Enrichr?

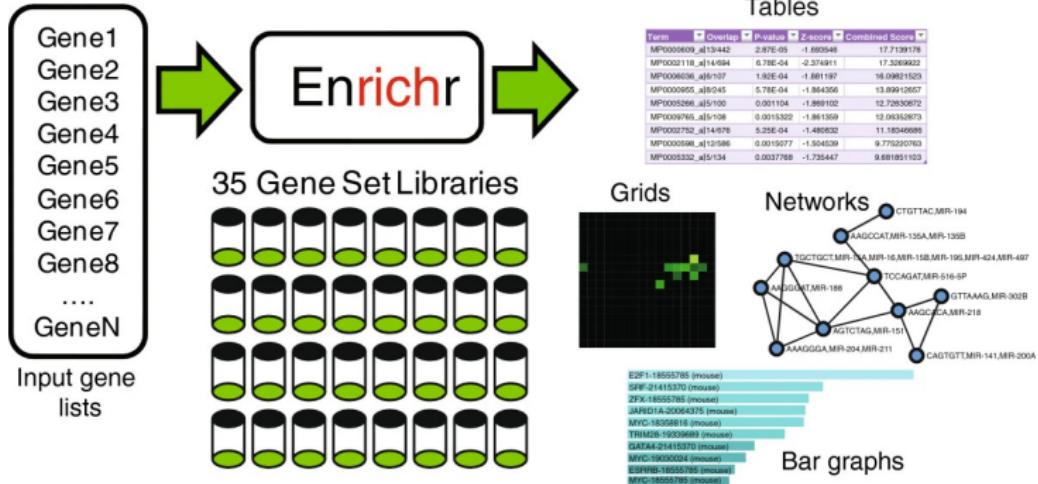


Figure: Enrichr workflow (E. Y. Chen et al., 2013; Kuleshov et al., 2016)

# Gene-set Library

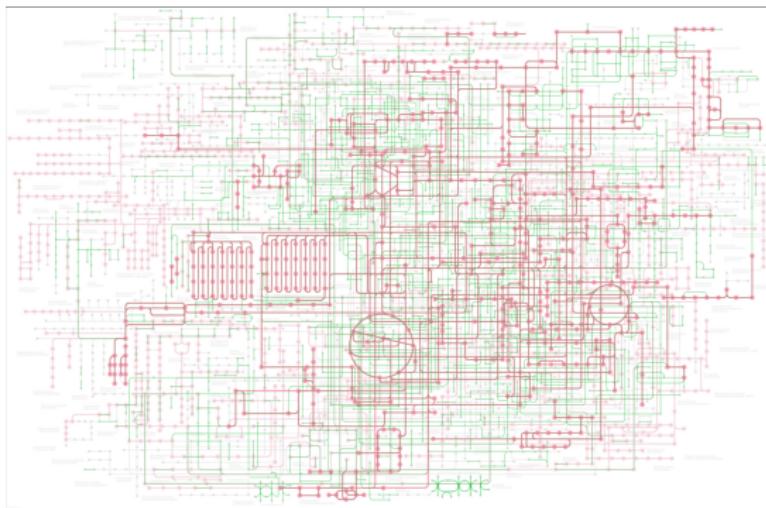


Figure: The global map of metabolic pathways by KEGG (Kanehisa et al., 2021)

KEGG

KEGG 2021 Human

# WTS Data Composition

Table: Number of WTS samples

Cancer Subtype	Stage	Number of Samples	
		Normal	Dysplasia
LUSC	Normal	17	
	Dysplasia		2
	CIS+AIS	34	
	Primary	36	
	Total	89	
LUAD	Normal	13	
	AAH		1
	CIS+AIS	5	
	Primary	6	
	Total	25	

# WTS Data Composition by Recur |

Table: Number of WTS LUSC samples

Recurrence?	Number of Samples	
	Stage	
Recurrence	Normal	1
	Dysplasia	1
	CIS+AIS	5
	Primary	6
	Total	13
Non-recurrence	Normal	16
	Dysplasia	1
	CIS+AIS	29
	Primary	30
	Total	76

# WTS Data Composition by Recur II

Table: Number of WTS LUAD samples

Recurrence?	Stage	Number of Samples	
		Normal	Total
Recurrence	Normal	2	
	CIS+AIS	1	
	Primary	1	
	Total	4	
Non-recurrence	Normal	11	
	AAH	1	
	CIS+AIS	4	
	Primary	5	
	Total	21	

# WTS Data Composition by Smoking I

Table: Number of WTS LUSC samples

Smoking?	Stage	Number of Samples	
		Normal	AIS
Never	Normal	1	
	CIS+AIS	1	
	Primary	2	
	Total	4	
Ex	Normal	8	
	Dysplasia	1	
	CIS+AIS	21	
	Primary	22	
	Total	52	
Current	Normal	8	
	Dysplasia	1	
	CIS+AIS	12	
	Primary	12	
	Total	33	

# WTS Data Composition by Smoking II

Table: Number of WTS LUAD samples

Smoking?	Stage	Number of Samples	
Never	Normal	10	
	AAH	1	
	CIS+AIS	3	
	Primary	4	
	Total	18	
Ex	Normal	3	
	CIS+AIS	1	
	Primary	1	
	Total	5	
Current	CIS+AIS	1	
	Primary	1	
	Total	2	

## 4. Results

### 4.7. Differences in Gene Expression Levels

#### 4.7.1. Comparing cancer stage in LUSC

# DEG List in LUSC

Table: Up-regulated DEG in LUSC

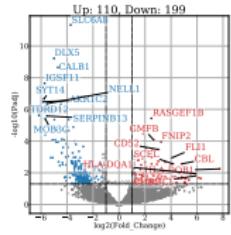
gene	log2FoldChange	pvalue	padj
AKR1C1	6.18e+00	5.14e-26	5.01e-23
AKR1C2	6.06e+00	1.19e-22	5.04e-20
CYP4F11	5.58e+00	1.51e-20	4.36e-18

Table: Down-regulated DEG in LUSC

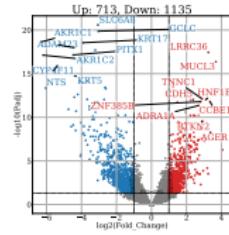
gene	log2FoldChange	pvalue	padj
SFTPC	-5.85e+00	9.16e-21	2.83e-18
FAM107A	-4.62e+00	2.27e-33	9.60e-30
LRRC36	-4.53e+00	5.49e-36	3.48e-32

## DEG Volcano Plots in LUSC

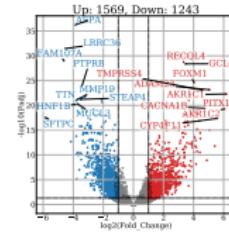
Normal → Dysplasia → CIS → Primary (LUSC)



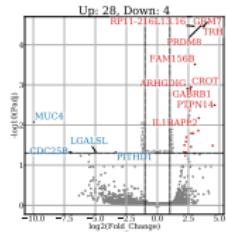
(a) Normal-Dysplasia



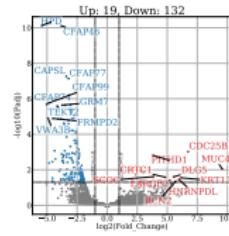
(b) Normal-CIS



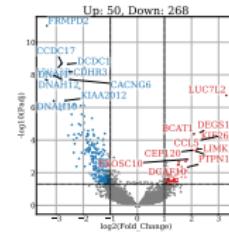
(c) Normal-Primary



(d) Dysplasia-CIS



(e) Dysplasia-Primary

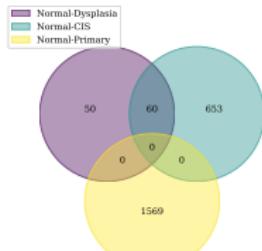


(f) CIS-Primary

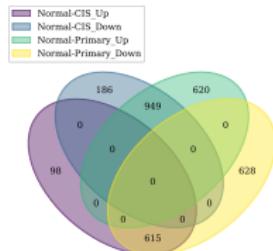
## Figure: DEG Volcano Plots in LUSC

# DEG Venn Diagram in LUSC

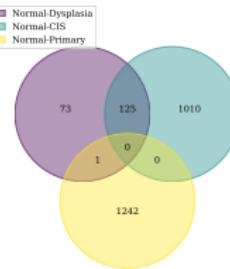
Normal → Dysplasia → CIS → Primary (LUSC)



(a) Up-regulated



(b) Both



(c) Down-regulated

Figure: DEG Venn Diagram in LUSC

# Enrichment test with Normal vs. Dysplasia in LUSC

Table: Up-regulated Pathways on Normal vs. Dysplasia

Term name (19)	Overlapping genes...	Adjusted p-value
Leishmaniasis	NFKBIA,HLA-DOA,TLR4,...(5)	6.72e-03
Lysosome	ASAHI,LAPTM5,CTSH,...(6)	6.72e-03
Phagosome	OLR1,HLA-DOA,TLR4,...(6)	1.15e-02

Table: Down-regulated Pathways on Normal vs. Dysplasia

Term name	Overlapping genes...	Adjusted p-value
None		

# Enrichment test with Normal vs. CIS in LUSC

Table: Up-regulated Pathways on Normal vs. CIS

Term name (50)	Overlapping genes...	Adjusted p-value
Hematopoietic cell lineage	CSF1R,CSF3,IL4R,...(20)	7.22e-08
Malaria	CSF3,HGF,ITGB2,...(13)	1.16e-06
Cell adhesion molecules	NLGN3,SELPLG,CADM1,...(22)	1.16e-06

Table: Down-regulated Pathways on Normal vs. CIS

Term name (21)	Overlapping genes...	Adjusted p-value
Metabolism of xenobiotics by cytochrome P450	GSTM4,CBR1,GSTM3,...(19)	9.34e-06
Drug metabolism	GSTM4,GSTM3,GSTM2,...(21)	9.06e-05
Cell cycle	CDKN2A,PLK1,BUB1B,...(22)	1.68e-04

# Enrichment test with Normal vs. Primary in LUSC

Table: Up-regulated Pathways on Normal vs. Primary

Term name (30)	Overlapping genes...	Adjusted p-value
Cell cycle	HDAC1,PKMYT1,ORC4,...(27)	1.53e-04
Glutathione metabolism	GSTM4,GSTM3,G6PD,...(17)	1.53e-04
DNA replication	FEN1,RNASEH2A,RFC4,...(13)	1.72e-04

Table: Down-regulated Pathways on Normal vs. Primary

Term name (53)	Overlapping genes...	Adjusted p-value
Hematopoietic cell lineage	CSF1R,CSF3,CSF3R,...(27)	7.33e-09
Malaria	IL10,CSF3,CR1,...(19)	7.33e-09
Hypertrophic cardiomyopathy	LAMA2,ITGB3,CACNA1D,...(25)	1.24e-08

# Findings in Comparing cancer stage in LUSC

## AKR1C1 & AKR1C2

- ① Down-regulated in CIS, but up-regulated in Primary.
- ② Regulate steroids (Jin et al., 2009) and hormones (Penning et al., 2000).
- ③ Promote the metastasis of NSCLC (Z. Hong et al., 2018).

## SFTPC

- ① Down-regulate in Primary than Normal.
- ② A pulmonary surfactant associated protein (Lin et al., 2018).
- ③ SFTPC  $\downarrow \Rightarrow$  Poor survival in LUAD (Li et al., 2019).
- ④ Associated with lung disease in adult (Henderson et al., 2013) and baby (Brasch et al., 2004).

## 4. Results

### 4.7. Differences in Gene Expression Levels

#### 4.7.2. Comparing cancer stage in LUAD

# DEG List in LUSC

Table: Up-regulated DEG in LUAD

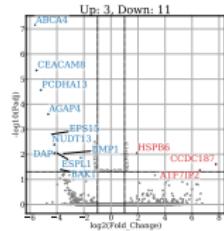
gene	log2FoldChange	pvalue	padj
ABCA4	4.95e+00	3.01e-12	2.58e-09
HMGA2	4.79e+00	8.06e-08	1.46e-05
KIF12	4.48e+00	1.33e-06	1.46e-04

Table: Down-regulated DEG in LUAD

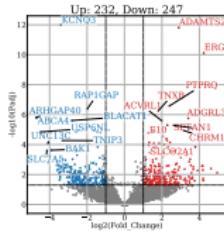
gene	log2FoldChange	pvalue	padj
SLC6A4	-6.20e+00	5.80e-10	2.36e-07
IL1RL1	-4.20e+00	7.47e-06	5.82e-04
RNF185	-4.06e+00	4.75e-05	2.45e-03

## DEG Volcano Plots in LUAD

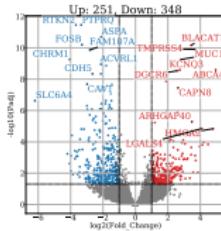
Normal → AAH → AIS → Primary (LUAD)



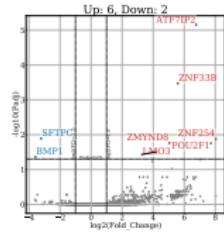
(a) Normal-AAH



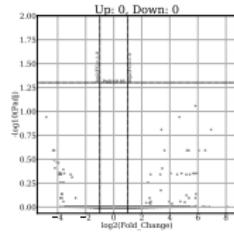
(b) Normal-AIS



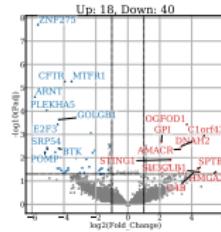
(c) Normal-Primary



(d) AAH-AIS



(e) AAH-Primary

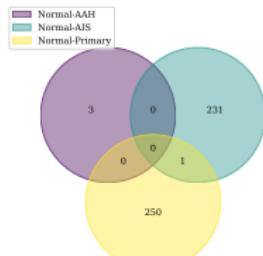


(f) AIS-Primary

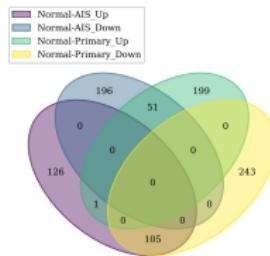
## Figure: DEG Volcano Plots in LUAD

# DEG Venn Diagram in LUAD

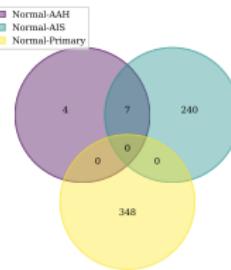
Normal → AAH → AIS → Primary (LUAD)



(a) Up-regulated



(b) Both



(c) Down-regulated

Figure: DEG Venn Diagram in LUAD

## Enrichment test with Normal vs. AAH in LUAD

Table: Up-regulated Pathways on Normal vs. AAH

Term name	Overlapping genes...	Adjusted p-value
None		

Table: Down-regulated Pathways on Normal vs. AAH

Term name	Overlapping genes...	Adjusted p-value
None		

# Enrichment test with Normal vs. AIS in LUAD

Table: Up-regulated Pathways on Normal vs. AIS

Term name	Overlapping genes...	Adjusted p-value
Calcium signaling pathway	RYR2,NTRK2,CHRM1,...(11)	2.49e-02
Cell adhesion molecules	CDH5,CLDN5,NLGN1,...(8)	3.55e-02

Table: Down-regulated Pathways on Normal vs. AIS

Term name	Overlapping genes...	Adjusted p-value
None		

# Enrichment test with Normal vs. Primary in LUAD

Table: Up-regulated Pathways on Normal vs. Primary

Term name	Overlapping genes...	Adjusted p-value
None		

Table: Down-regulated Pathways on Normal vs. Primary

Term name (15)	Overlapping genes...	Adjusted p-value
Vascular smooth muscle contraction	PPP1R14A,EDN1,RAMP2,...(13)	1.38e-04
ECM-receptor interaction	TNXB,VWF,COL4A2,...(10)	3.58e-04
Calcium signaling pathway	MCOLN3,CHRM1,NOS2,...(16)	4.03e-04

## ABCA4

- ① Down-regulated in AAH & AIS, but up-regulated in Primary.
- ② It is associated with ophthalmology (Maugeri et al., 2000).
- ③ It shows lung cancer susceptibility in Korean patients (Lee, Lee, Yoon, & Lee, 2013).

# Finding in Comparing cancer stage in LUAD II

## KCNQ3

- ① Down-regulated in AIS, but up-regulated in Primary.
- ②  $K^+$  voltage-dependent channels  $\Rightarrow$  Various physiological functions (Schroeder, Kubisch, Stein, & Jentsch, 1998; Surti, Huang, Jan, Jan, & Cooper, 2005; Singh et al., 2003).
- ③ Up-regulated microRNAs in hypoxia-induced LUAD (Geng et al., 2016).
- ④ KCNQ gene family is associated with lung diseases (Mondejar-Parreño, Perez-Vizcaino, & Cogolludo, 2020).

## CHRM1

- ① Up-regulated in AIS, but down-regulated in Primary.
- ② Various cellular responses ⇒ neurodevelopmental disorders (Marcé-Grau et al., 2021), schizophrenia (Dean & Scarr, 2021), and Alzheimer's disease (Counts et al., 2007).
- ③ Reported down-regulation in LUSC & LUAD (Ma et al., 2019).

## 4. Results

### 4.7. Differences in Gene Expression Levels

#### 4.7.3. Recur vs. Non-recur in LUSC

Table: Number of WTS LUSC samples

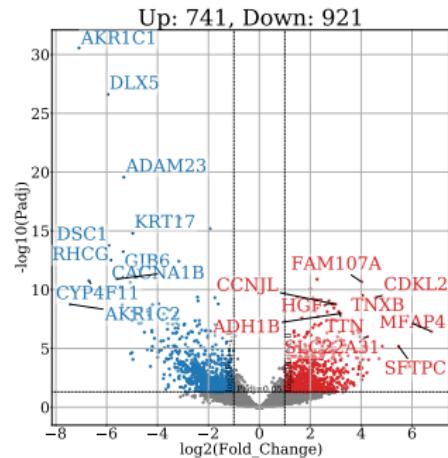
Recurrence?	Stage	Number of Samples	
		Normal	Dysplasia
Recurrence	Normal	1	
	Dysplasia		1
	CIS+AIS	5	
	Primary	6	
	Total	13	
Non-recurrence	Normal	16	
	Dysplasia		1
	CIS+AIS	29	
	Primary	30	
	Total	76	

## Pooled normal samples

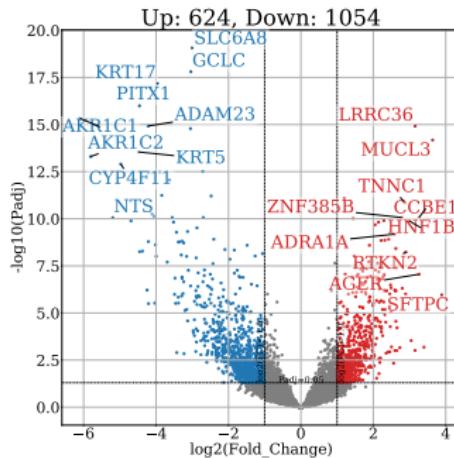
In order to compare with Normal stage, merging Normal samples.

∴ Insufficient number of Normal samples in Recur.

# DEG Volcano Plots for R vs. NR with CIS in LUSC



(a) Recur



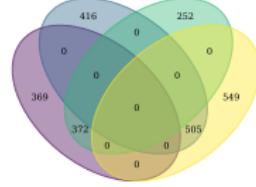
(b) Non-recur

Figure: DEG Volcanot Plot with CIS in LUSC

# DEG Venn Diagram for R vs. NR with CIS in LUSC



(a) Up-regulated



(b) Both



(c) Down-regulated

Figure: DEG Venn Diagram for R vs. NR with CIS in LUSC

# Enrichment test for Recur-specific with CIS in LUSC

Table: Up-regulated Pathways for Recur-specific

Term name	Overlapping genes...	Adjusted p-value
None		

Table: Down-regulated Pathways for Recur-specific

Term name (8)	Overlapping genes...	Adjusted p-value
Huntington disease	COX8A,DCTN5,COX7B,...(24)	6.36e-06
Amyotrophic lateral sclerosis	DCTN5,COX7B,TOMM40,...(25)	1.62e-05
Parkinson disease	COX8A,COX7B,NDUFA12,...(20)	1.62e-05

# Enrichment test for Non-recur-specific with CIS in LUSC

Table: Up-regulated Pathways for Non-recur-specific

Term name (4)	Overlapping genes...	Adjusted p-value
Malaria	IL6,ITGB2,KLRC4-KLRK1,...(6)	7.76e-03
Th1 and Th2 cell differentiation	STAT5B,MAML2,MAML3,...(7)	1.15e-02
Transcriptional misregulation in cancer	PTCRA,CSF1R,IL6,...(10)	1.15e-02

Table: Down-regulated Pathways for Non-recur-specific

Term name	Overlapping genes...	Adjusted p-value
None		

# Enrichment test for Intersected with CIS in LUSC

Table: Up-regulated Pathways for Intersected

Term name (41)	Overlapping genes...	Adjusted p-value
Hypertrophic cardiomyopathy	EDN1,CACNB4,ACE,...(12)	1.44e-05
Cell adhesion molecules	CADM1,ICAM2,SELP,...(15)	1.44e-05
Hematopoietic cell lineage	CSF3,HLA-DMA,MME,...(12)	2.57e-05

Table: Down-regulated Pathways for Intersected

Term name (23)	Overlapping genes...	Adjusted p-value
Metabolism of xenobiotics by cytochrome P450	GSTM4,CBR1,GSTM3,...(14)	1.53e-06
Drug metabolism	GSTM4,GSTM3,RRM1,...(16)	1.69e-06
Steroid hormone biosynthesis	UGT1A1,SRD5A1,HSD17B1,...(11)	2.77e-05

# DEG Volcano Plots for R vs. NR with Primary in LUSC

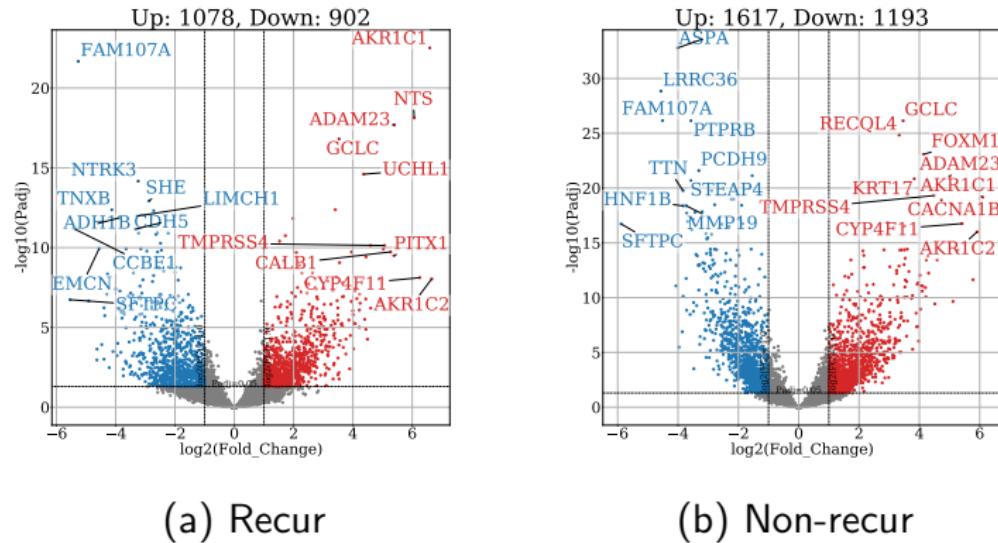
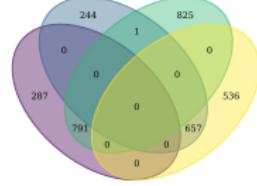


Figure: DEG Volcanot Plot with Primary in LUSC

# DEG Venn Diagram for R vs. NR with Primary in LUSC



(a) Up-regulated



(b) Both



(c) Down-regulated

Figure: DEG Venn Diagram for R vs. NR with Primary in LUSC

# Enrichment test for Recur-specific with Primary in LUSC

Table: Up-regulated Pathways for Recur-specific

Term name (9)	Overlapping genes...	Adjusted p-value
Amyotrophic lateral sclerosis	COX7B,HSPA5,NDUFA1,...(17)	4.85e-03
RNA transport	NUP93,EIF5,EIF1AY,...(11)	6.11e-03
mRNA surveillance pathway	PABPN1,PPP2R1A,NCBP2,...(8)	6.11e-03

Table: Down-regulated Pathways for Recur-specific

Term name	Overlapping genes...	Adjusted p-value
None		

# Enrichment test for NR-specific with Primary in LUSC

Table: Up-regulated Pathways for Non-recur-specific

Term name	Overlapping genes...	Adjusted p-value
Homologous recombination	RAD51D,POLD1,RPA3,...(9)	1.00e-02

Table: Down-regulated Pathways for Non-recur-specific

Term name (19)	Overlapping genes...	Adjusted p-value
Staphylococcus aureus infection	IL10,CFD,ITGB2,...(14)	5.37e-05
Hematopoietic cell lineage	CR1,MME,ITGB3,...(14)	5.37e-05
Leishmaniasis	IL10,C3,NFKBIA,...(11)	4.30e-04

# Enrichment test for Intersected with Primary in LUSC

Table: Up-regulated Pathways for Intersected

Term name (36)	Overlapping genes...	Adjusted p-value
Glycolysis / Gluconeogenesis	GPI,TPI1,PDHA1,...(16)	1.09e-06
Drug metabolism	GSTM4,GSTM3,GSTM2,...(20)	1.09e-06
Metabolism of xenobiotics by cytochrome P450	GSTM4,CBR1,GSTM3,...(15)	2.27e-05

Table: Down-regulated Pathways for Intersected

Term name (23)	Overlapping genes...	Adjusted p-value
Dilated cardiomyopathy	LAMA2,TNNC1,ADCY4,...(15)	1.44e-04
Hypertrophic cardiomyopathy	LAMA2,TNNC1,CACNA2D2,...(14)	1.81e-04
Malaria	SELP,CSF3,IL6,...(10)	2.91e-04

# Finding in Comparing Recur vs. Non-recur in LUSC I

## NTS

- ① Highly up-regulated in Recur patients.
- ② Neurotensin.
- ③ Association with non-gastrointestinal cancers (Nikolaou et al., 2020).
- ④ Modulate lung cancer cell plasticity and heterogeneity (Wu et al., 2019).

## NTRK3

- ① Highly down-regulated in Recur patients.
- ② Activation of NTRK3 in LUSC (Bollig-Fischer et al., 2021).
- ③ NTRK3 mutation has association with immunotherapy in LUAD (Niu et al., 2020).

# Finding in Comparing Recur vs. Non-recur in LUSC II

## RECQL4

- ① Highly up-regulated in Non-recur patients.
- ② DNA-dependent ATPase (Yin, Kwon, Varshavsky, & Wang, 2004)
- ③ RECQL4 modulate chromosome segregation (Yin et al., 2004)
- ④ RECQL5 promotes metastasis & resistance in NSCLC (Xia, Zhang, Yuan, & Niu, 2021)
- ⑤ RECQL4 ↑ ⇒ Poor prognosis in breast cancer (Zhu et al., 2018)
  - ① Overall survival
  - ② Distant metastasis-free survival
  - ③ Relapse-free survival

## 4. Results

### 4.7. Differences in Gene Expression Levels

#### 4.7.4. Within Recur in LUSC

# DEG List for CIS within Recur in LUSC

Table: Up-regulated DEG for CIS within Recur in LUSC

gene	log2FoldChange	pvalue	padj
MFAP4	6.77e+00	2.70e-09	3.72e-07
TBX2	5.90e+00	1.19e-05	3.40e-04
SFTPC	5.47e+00	8.66e-08	6.57e-06

Table: Down-regulated DEG for CIS within Recur in LUSC

gene	log2FoldChange	pvalue	padj
AKR1C2	-7.44e+00	4.70e-12	1.74e-09
AKR1C1	-7.09e+00	2.35e-35	2.74e-31
CYP4F11	-6.70e+00	1.95e-14	1.75e-11

# DEG List for Primary within Recur in LUSC

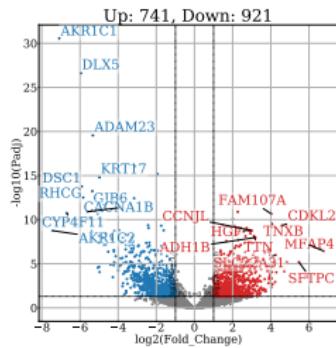
Table: Up-regulated DEG for Primary within Recur in LUSC

gene	log2FoldChange	pvalue	padj
AKR1C2	6.66e+00	4.34e-11	9.07e-09
AKR1C1	6.59e+00	2.62e-27	3.06e-23
CYP4F11	6.25e+00	3.61e-11	7.67e-09

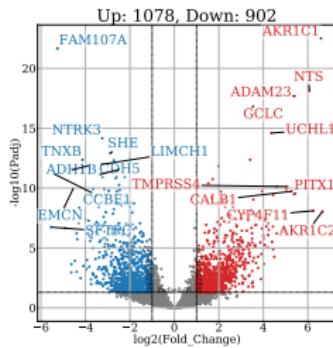
Table: Down-regulated DEG for Primary within Recur in LUSC

gene	log2FoldChange	pvalue	padj
SFTPC	-5.54e+00	1.56e-09	1.83e-07
CCBE1	-5.36e+00	9.73e-15	7.11e-12
FAM107A	-5.27e+00	3.64e-26	2.13e-22

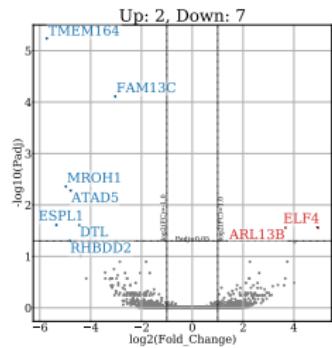
# DEG Volcano Plots with Recur in LUSC



(a) Normal-CIS



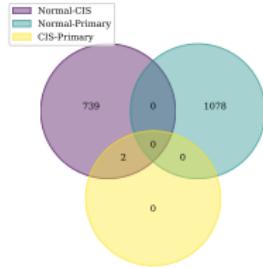
(b) Normal-Primary



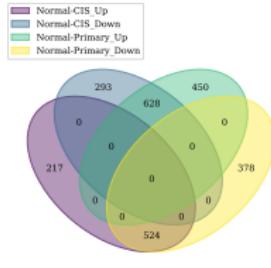
(c) CIS-Primary

Figure: DEG Volcano Plots with Recur samples in LUSC

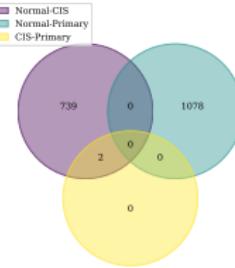
# DEG Venn Diagram with Recur in LUSC



(a) Up-regulated



(b) Both



(c) Down-regulated

Figure: DEG Venn Diagram with Recur samples in LUSC

## Enrichment test with Normal vs. CIS for Recur

Table: Up-regulated Pathways on Normal vs. CIS for Recur in LUSC

Term name (40)	Overlapping genes...	Adjusted p-value
Hematopoietic cell lineage	CSF3,CSF3R,IL4R,...(17)	1.87e-05
Cell adhesion molecules	SELPLG,CADM1,SDC3,...(21)	1.87e-05
Hypertrophic cardiomyopathy	EDN1,ACE,TNNC1,...(15)	9.66e-05

Table: Down-regulated Pathways on Normal vs. CIS for Recur in LUSC

Term name (31)	Overlapping genes...	Adjusted p-value
Parkinson disease	COX7B,NDUFA12,NDUFB5,...(32)	2.11e-05
Alzheimer disease	COX7B,NDUFA12,NDUFB5,...(41)	2.11e-05
Huntington disease	DCTN5,COX7B,NDUFA12,...(36)	2.11e-05

# Enrichment test with Normal vs. Primary for Recur

Table: Up-regulated Pathways on Normal vs. Primary for Recur in LUSC

Term name (39)	Overlapping genes...	Adjusted p-value
Glycolysis / Gluconeogenesis	GPI,TPI1,PDHA1,...(17)	1.90e-05
RNA transport	EIF4A2,NUP205,NUP62,...(29)	2.66e-05
Drug metabolism	GSTM4,GSTM3,GSTM2,...(21)	2.66e-05

Table: Down-regulated Pathways on Normal vs. Primary for Recur in LUSC

Term name (14)	Overlapping genes...	Adjusted p-value
Dilated cardiomyopathy	LAMA2,ITGA3,TNNC1,...(20)	2.19e-06
Hypertrophic cardiomyopathy	LAMA2,ITGA3,TNNC1,...(19)	2.19e-06
Arrhythmogenic right ventricular cardiomyopathy	TCF7L2,LAMA2,ACTN2,...(17)	4.12e-06

# Finding in Comparing within Recur in LUSC I

## AKR1C1

- ① Down-regulated in CIS, but up-regulated in Primary.
- ② Regulate steroids (Jin et al., 2009) and hormones (Penning et al., 2000).
- ③ Promote the metastasis of NSCLC (Z. Hong et al., 2018)

## ADAM23

- ① Down-regulated in CIS, but up-regulated in Primary.
- ② Play a role in cell-cell and cell-matrix interactions (Cal, Freije, López, Takada, & Lopez-Otin, 2000)
- ③ Suppresses metastasis in lung carcinoma cells (Ota et al., 2016)
- ④ ADAM protein was lower in NSCLC than in normal tissue & benign pulmonary lesions (Hu et al., 2011)

## FAM107A

- ① Up-regulated in CIS, but down-regulated in Primary.
- ② May play a role in tumor development (L. Wang et al., 2000)
- ③ Negatively regulates focal adhesion assembly (Le et al., 2010)

## 4. Results

### 4.7. Differences in Gene Expression Levels

#### 4.7.5. Within Non-recur in LUSC

# DEG List for CIS within Non-recr in LUSC

Table: Up-regulated DEG for CIS within Non-recr in LUSC

gene	log2FoldChange	pvalue	padj
SFTPC	3.89e+00	1.33e-08	1.10e-06
MUCL3	3.64e+00	4.99e-18	6.71e-15
HLA-DRB1	3.40e+00	3.00e-05	6.50e-04

Table: Down-regulated DEG for CIS within Non-recr in LUSC

gene	log2FoldChange	pvalue	padj
AKR1C1	-6.10e+00	1.95e-19	4.73e-16
AKR1C2	-5.81e+00	4.57e-17	5.03e-14
NTS	-5.19e+00	1.60e-13	8.61e-11

# DEG List for Primary within Non-recur in LUSC

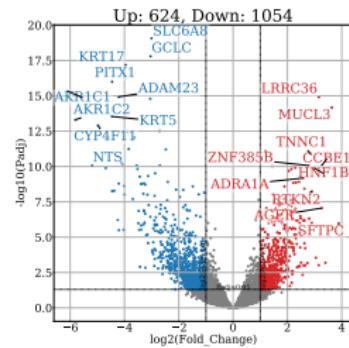
Table: Up-regulated DEG for Primary within Non-recur in LUSC

gene	log2FoldChange	pvalue	padj
AKR1C1	6.10e+00	9.04e-23	6.57e-20
AKR1C2	5.91e+00	3.13e-19	9.92e-17
NTS	5.78e+00	2.01e-14	2.28e-12

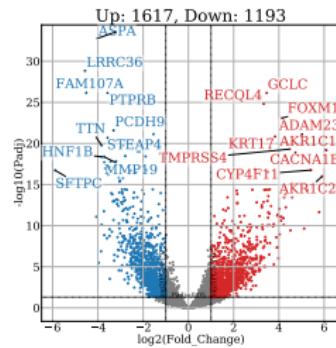
Table: Down-regulated DEG for Primary within Non-recur in LUSC

gene	log2FoldChange	pvalue	padj
SFTPC	-5.89e+00	5.10e-20	1.86e-17
LRRC36	-4.57e+00	2.29e-33	1.42e-29
FAM107A	-4.51e+00	2.49e-30	7.01e-27

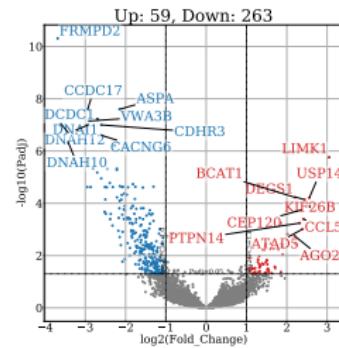
# DEG Volcano Plots with Non-recr in LUSC



(a) Normal-CIS



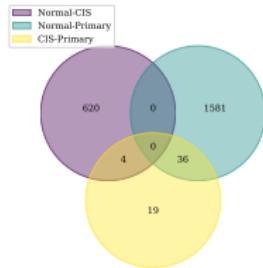
(b) Normal-Primary



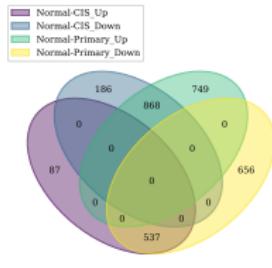
(c) CIS-Primary

Figure: DEG Volcano Plots with Non-recr samples in LUSC

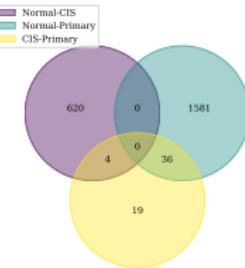
# DEG Venn Diagram with Non-recur in LUSC



(a) Up-regulated



(b) Both



(c) Down-regulated

Figure: DEG Venn Diagram with Non-recur in LUSC

## Enrichment test with Normal vs. CIS for Non-recur

Table: Up-regulated Pathways on Normal vs. CIS for Non-recur in LUSC

Term name (48)	Overlapping genes...	Adjusted p-value
Malaria	CSF3,HGF,ITGB2,...(14)	6.53e-08
Hematopoietic cell lineage	CSF1R,CSF3,MME,...(18)	2.01e-07
Hypertrophic cardiomyopathy	EDN1,ACE,LAMA2,...(16)	1.53e-06

Table: Down-regulated Pathways on Normal vs. CIS for Non-recur in LUSC

Term name (20)	Overlapping genes...	Adjusted p-value
Metabolism of xenobiotics by cytochrome P450	GSTM4,CBR1,GSTM3,...(17)	9.67e-05
Drug metabolism	GSTM4,GSTM3,RRM1,...(20)	1.18e-04
Cell cycle	GADD45A,CDKN2A,PLK1,...(21)	1.89e-04

## Enrichment test with Normal vs. Primary for Non-recur

**Table:** Up-regulated Pathways on Normal vs. Primary for Non-recur in LUSC

Term name (30)	Overlapping genes...	Adjusted p-value
Cell cycle	HDAC1,PKMYT1,ORC5,...(31)	3.04e-06
DNA replication	FEN1,RNASEH2A,RFC4,...(15)	6.47e-06
Homologous recombination	BLM,RPA1,PALB2,...(15)	3.33e-05

**Table:** Down-regulated Pathways on Normal vs. Primary for Non-recur in LUSC

Term name (57)	Overlapping genes...	Adjusted p-value
Hematopoietic cell lineage	CSF1R,CSF3,CSF3R,...(28)	6.65e-10
Malaria	IL10,CSF3,CR1,...(19)	3.57e-09
Hypertrophic cardiomyopathy	LAMA2,ITGB3,CACNA1D,...(25)	5.12e-09

# Finding in Comparing within Non-recur in LUSC I

## AKR1C1 & AKR1C2

- ① Down-regulated in CIS, but up-regulated in Primary.
- ② Regulate steroids (Jin et al., 2009) and hormones (Penning et al., 2000)
- ③ Promote the metastasis of NSCLC (Z. Hong et al., 2018)

## CYP4F11

- ① Down-regulated in CIS, but up-regulated in Primary.
- ② Involved in the metabolism, including fatty acid and their derivatives (Edson et al., 2013; Kalsotra, Turman, Kikuta, & Strobel, 2004; Dhar, Sepkovic, Hirani, Magnusson, & Lasker, 2008)
- ③ CYP4F11 showed a strong association with survival in colorectal cancer (Alnabulsi, Swan, Cash, Alnabulsi, & Murray, 2017).

## LRRC36

- ① Up-regulated in CIS, but down-regulated in Primary.
- ② Leucine-rich repeat-containing protein 36
- ③ LRRC36 is positively correlated with survival in LUAD (Zhang et al., 2017).

## 4. Results

### 4.7. Differences in Gene Expression Levels

#### 4.7.6. Within Non-recur in LUAD

# LUAD Data Composition

Table: Number of WTS LUAD samples

Recurrence?	Stage	Number of Samples	
Recurrence	Normal	2	
	CIS+AIS	1	
	Primary	1	
	Total	4	
Non-recurrence	Normal	11	
	AAH	1	
	CIS+AIS	4	
	Primary	5	
	Total	21	

# DEG List for AIS within Non-recur in LUAD

Table: Up-regulated DEG for AIS within Non-recur in LUAD

gene	log2FoldChange	pvalue	padj
MUC4	4.83e+00	2.55e-04	1.68e-02
SIPA1	4.77e+00	4.87e-05	6.37e-03
C11orf45	4.68e+00	2.86e-04	1.85e-02

Table: Down-regulated DEG for AIS within Non-recur in LUAD

gene	log2FoldChange	pvalue	padj
ABCA4	-5.02e+00	2.44e-10	5.29e-07
UNC13C	-4.08e+00	6.49e-06	1.88e-03
SLC7A5	-3.93e+00	1.40e-06	6.76e-04

# DEG List for Primary within Non-recur in LUAD

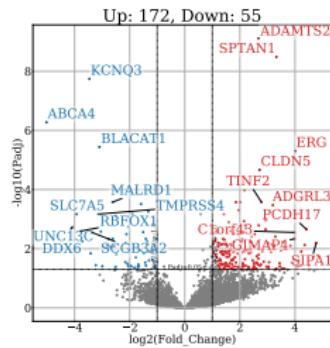
Table: Up-regulated DEG for Primary within Non-recur in LUAD

gene	log2FoldChange	pvalue	padj
ABCA4	5.22e+00	1.67e-11	3.32e-08
HMGA2	5.03e+00	4.39e-07	9.62e-05
KIF12	4.54e+00	2.62e-06	3.91e-04

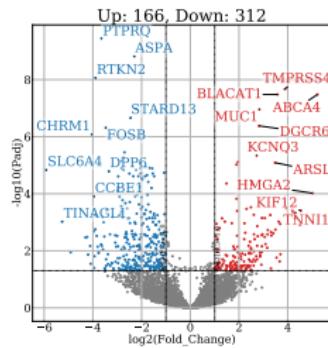
Table: Down-regulated DEG for Primary within Non-recur in LUAD

gene	log2FoldChange	pvalue	padj
SLC6A4	-5.92e+00	3.83e-08	1.47e-05
TINAGL1	-5.27e+00	9.47e-06	9.57e-04
SFTPA1	-4.91e+00	2.69e-04	1.13e-02

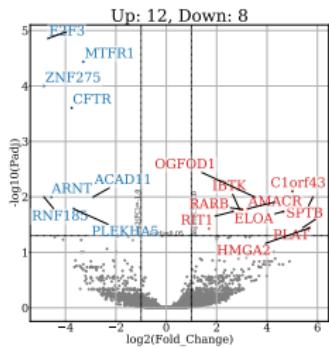
# DEG Volcano Plots with Non-recr in LUAD



(a) Normal-AIS



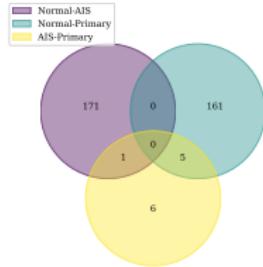
(b) Normal-Primary



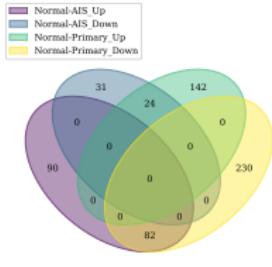
(c) AIS-Primary

Figure: DEG Volcano Plots with Non-recr samples in LUAD

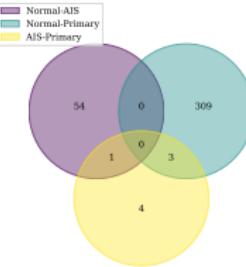
# DEG Venn Diagram with Non-recur in LUAD



(a) Up-regulated



(b) Both



(c) Down-regulated

Figure: DEG Venn Diagram with Non-recur in LUAD

# Enrichment test with Normal vs. AIS in LUAD

Table: Up-regulated Pathways on Normal vs. AIS for Non-recur in LUAD

Term name	Overlapping genes...	Adjusted p-value
Calcium signaling pathway	NTRK2, RYR2, CHRM1, ... (9)	3.90e-02

Table: Down-regulated Pathways on Normal vs. AIS for Non-recur in LUAD

Term name	Overlapping genes...	Adjusted p-value
None		

## Enrichment test with Normal vs. Primary in LUAD

Table: Up-regulated Pathways on Normal vs. Primary for Non-recur in LUAD

Term name	Overlapping genes...	Adjusted p-value
None		

Table: Down-regulated Pathways on Normal vs. Primary for Non-recur in LUAD

Term name (5)	Overlapping genes...	Adjusted p-value
ECM-receptor interaction	TNXB,VWF,COL4A2,...(9)	2.05e-03
Vascular smooth muscle contraction	PPP1R14A,EDN1,AGTR1,...(10)	4.98e-03
Calcium signaling pathway	MCOLN3,CHRM1,NOS2,...(13)	7.82e-03

## KCNQ3

- ① Down-regulated in AIS, but up-regulated in Primary.
- ②  $K^+$  voltage-dependent channels  $\Rightarrow$  Various physiological functions (Schroeder et al., 1998; Surti et al., 2005; Singh et al., 2003)
- ③ Up-regulated microRNAs in hypoxia-induced LUAD (Geng et al., 2016)
- ④ KCNQ gene family is associated with lung diseases (Mondejar-Parreño et al., 2020)

## BLACAT1

- ① Down-regulated in AIS, but up-regulated in Primary.
- ② Bladder cancer-associated transcript 1
- ③ Chemo-resistance of NSCLC (Huang et al., 2019)
- ④ Predicts poor prognosis in SCLC (W. Chen et al., 2019)
- ⑤ Up-regulated in many human cancers (Ye, Yang, Liu, Lv, & Ye, 2020)

# Findings in DEG Analysis

## 4. Results

### 4.8. Bulk Cell Deconvolution

# BisqueRNA?

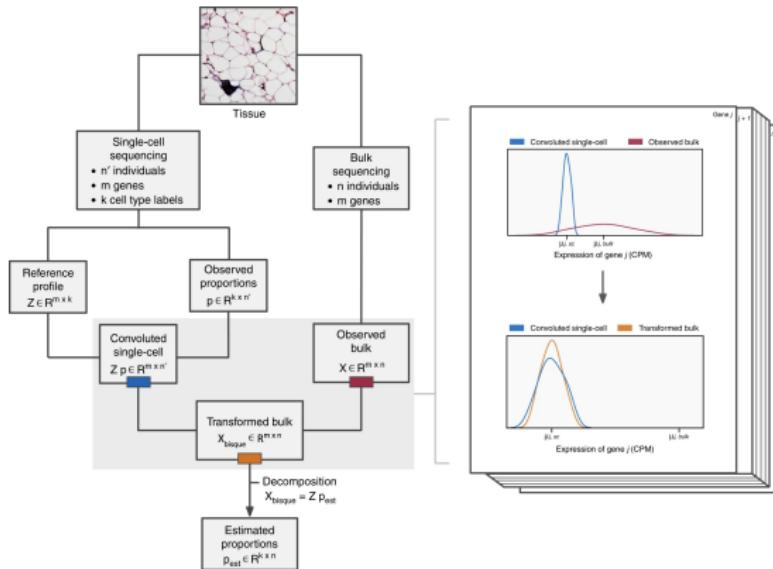


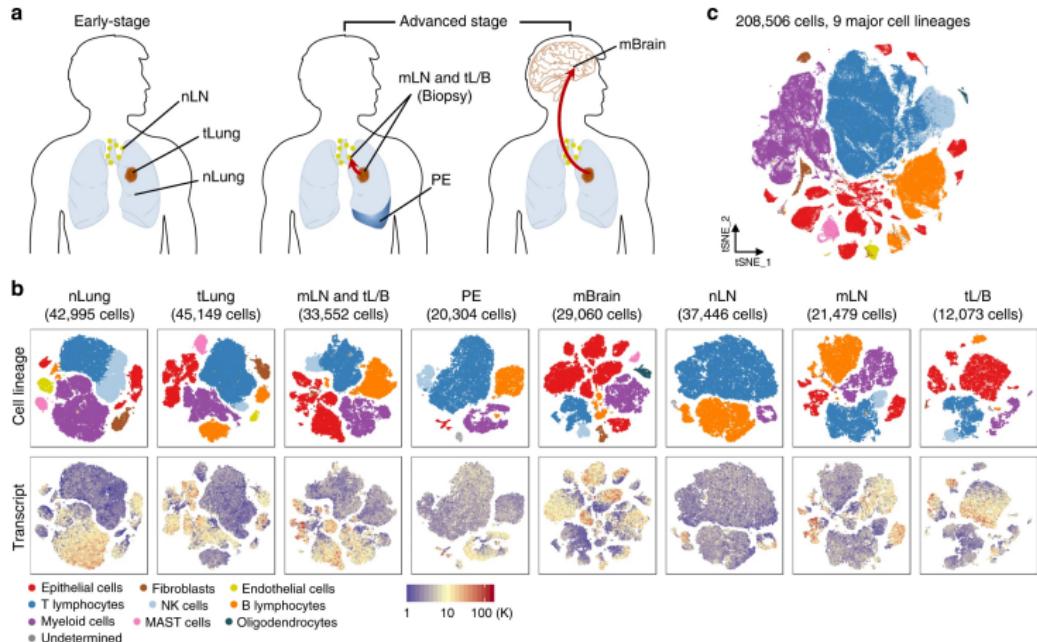
Figure: Workflow for BisqueRNA (Jew et al., 2020)

## 4. Results

### 4.8. Bulk Cell Deconvolution

#### 4.8.1. Reference by Kim et al. (2020)

# Reference Single-cell Data



**Figure:** Comprehensive dissection and clustering of 208,506 single cells from LUAD patients (Kim et al., 2020)

## Cluster Plot in LUSC

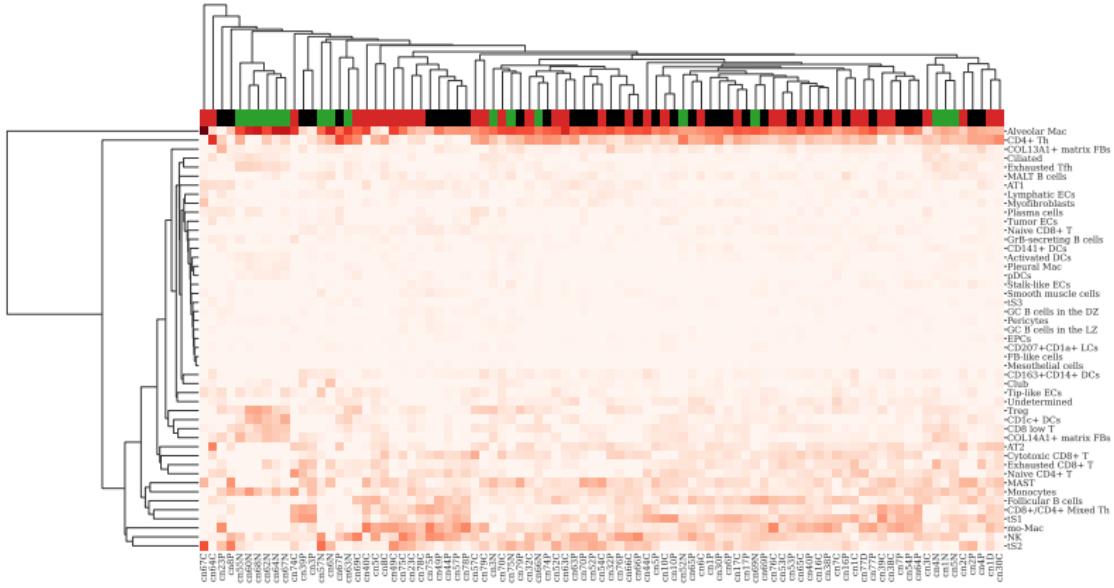
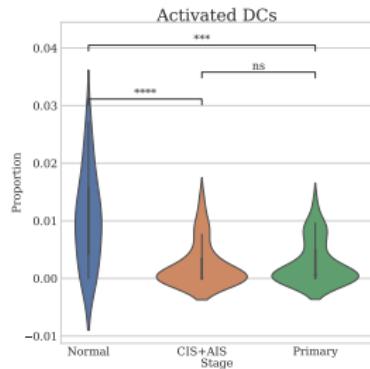
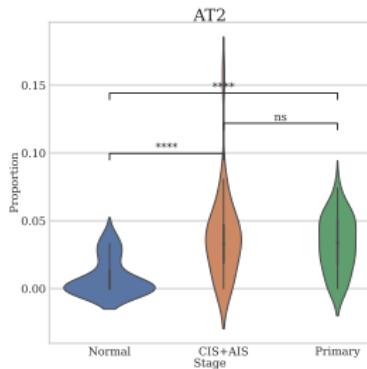


Figure: Cluster Plot in LUSC

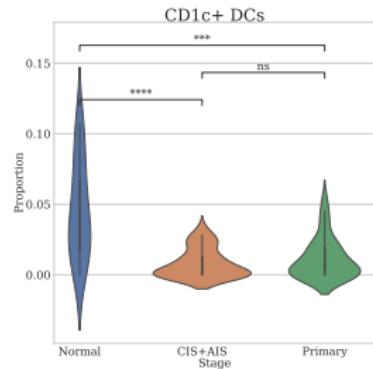
# Violin Plots in LUSC I



(a) Activated DCs



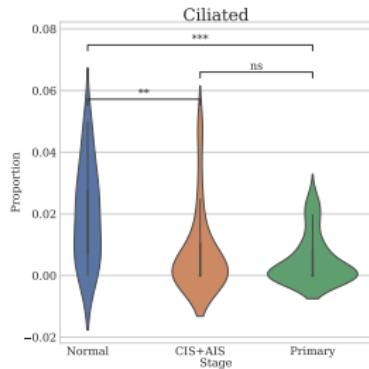
(b) Alveolar type II



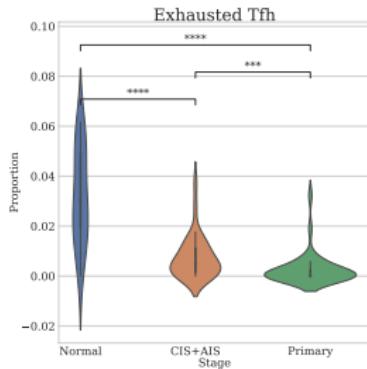
(c) Langerhans cells

Figure: Violin Plots in LUSC

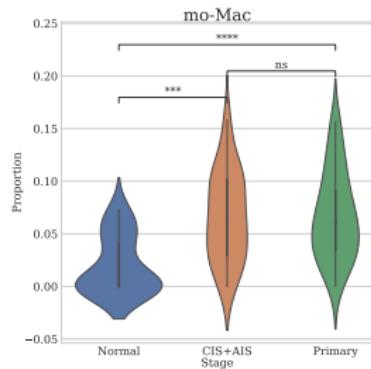
# Violin Plots in LUSC II



(d) Ciliated cells



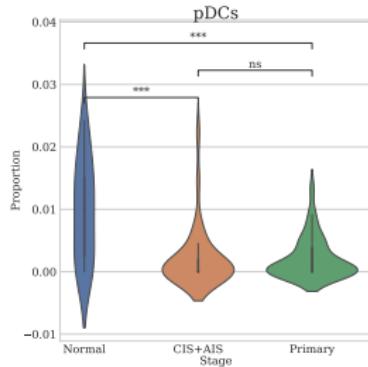
(e) Exhausted T follicular helper



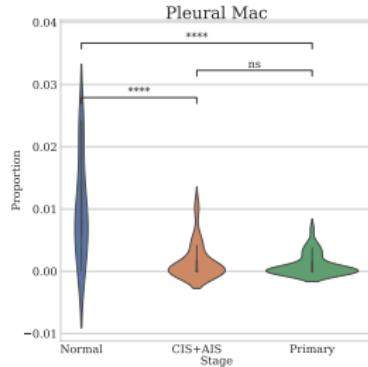
(f) Mo & Mac

Figure: Violin Plots in LUSC

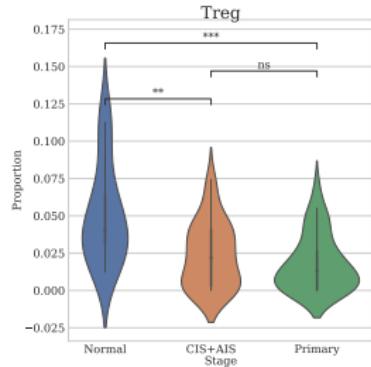
# Violin Plots in LUSC III



(g) Plasmacytoid DCs



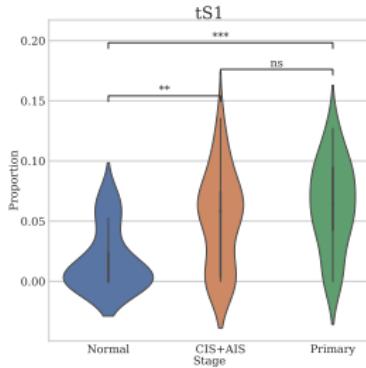
(h) Pleural Mac



(i) Regulatory T cells

Figure: Violin Plots in LUSC

# Violin Plots in LUSC IV



(j) Transcriptional states 1

Figure: Violin Plots in LUSC

# Findings in Bulk Cell Deconvolution with LUSC I

## Activated DCs

- ① Activated DCs have higher proportion in Normal samples.

## Alveolar type II

- ① Alveolar type II have lower proportion in Normal samples.

## CD1c+ DCs (Langerhans cells; LCs)

- ① LCs have higher proportion in Normal samples.

## Ciliated cells

- ① Ciliated cells have higher proportion in Normal samples.

# Findings in Bulk Cell Deconvolution with LUSC II

## Exhausted T follicular help cells

- ① Exhausted T follicular help cell is gradually decreased along cancer worsen.

## Monocyte & Macrophage

- ① Monocyte & Macrophage have lower proportion in Normal samples.

## Plasmacytoid DCs

- ① Plasmacytoid DCs have higher proportion in Normal samples.

## Pleural Macrophages

- ① Pleural Macrophages have higher proportion in Normal samples.

# Findings in Bulk Cell Deconvolution with LUSC III

## Regulatory T cells

- ① Regulatory T cells have higher proportion in Normal samples.

## Transcriptional states 1 (tS1)

- ① tS1 have lower proportion in Normal samples.

# Cluster Plot in LUAD

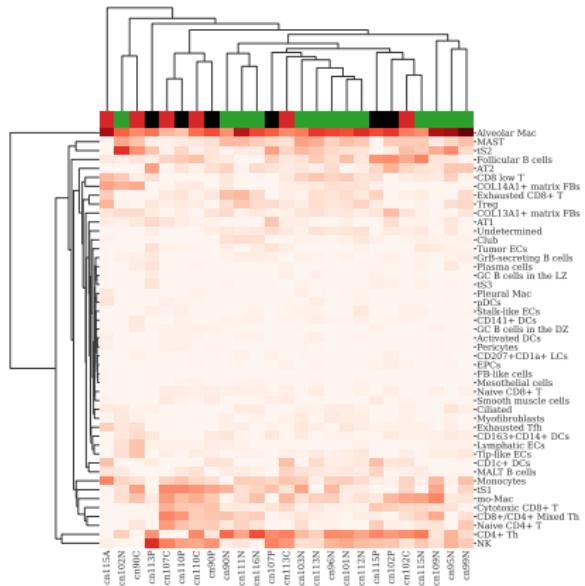
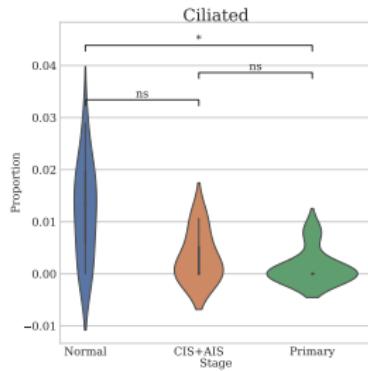
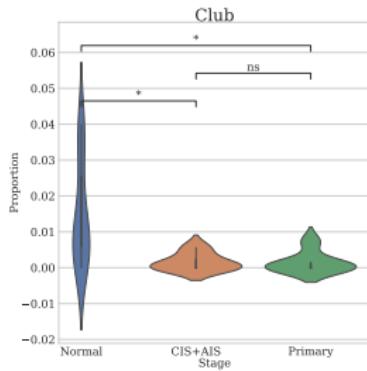


Figure: Cluster Plot in LUAD

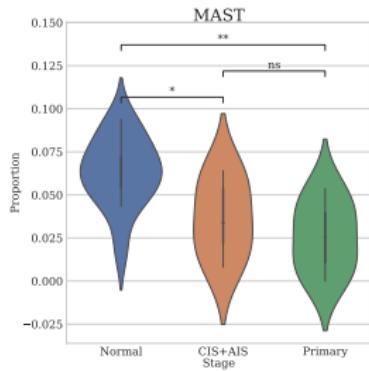
# Violin Plots in LUAD I



(a) Ciliated cells



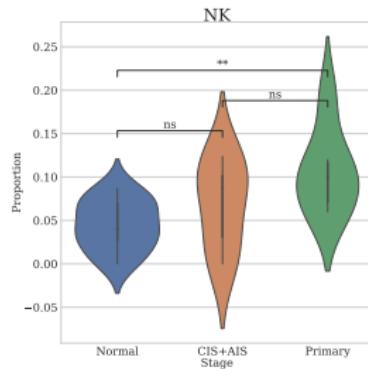
(b) Club Cell



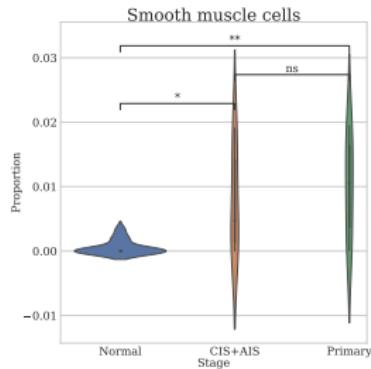
(c) Mast cell

Figure: Violin Plots in LUAD

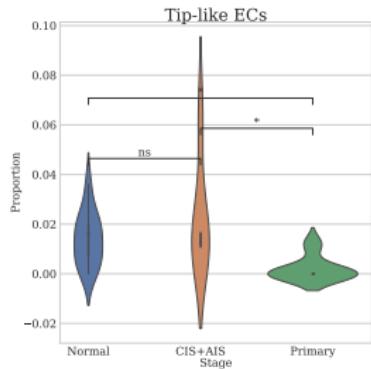
# Violin Plots in LUAD II



(d) NK cells



(e) Smooth muscle cells



(f) Tip-like ECs

Figure: Violin Plots in LUAD

# Findings in Bulk Cell Deconvolution with LUAD I

## Ciliated cells

- ① Ciliated cells have higher proportion in Normal than Primary samples.

## Club cells

- ① Club cells have higher proportion in Normal than Primary samples.

## Mast cells

- ① Mast cells have higher proportion in Normal than Primary samples.

## Natural Killer cells

- ① NK cells have higher proportion in Primary than Normal samples.

# Findings in Bulk Cell Deconvolution with LUAD II

## Smooth muscle cells

- ① Smooth muscle cells have higher proportion in Primary than Normal samples.

## Tip-like ECs

- ① Tip-like ECs have lower proportion in Primary than Normal samples.

## 4. Results

### 4.8. Bulk Cell Deconvolution

#### 4.8.2. Reference by Gueguen et al. (2021)

# Reference Single-cell Data

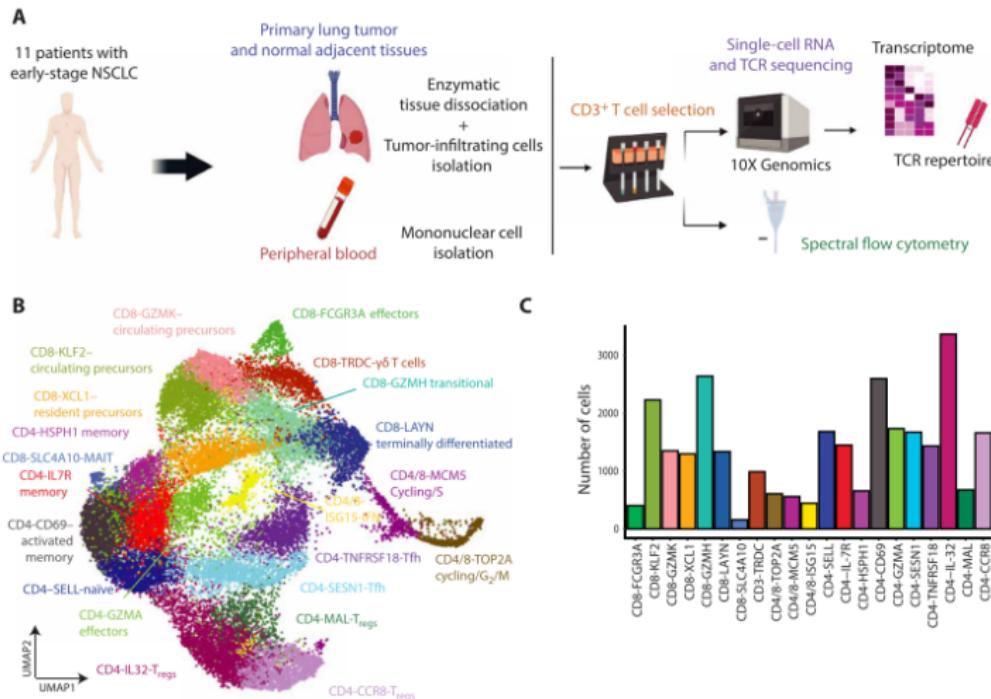


Figure: Characterization of CD3<sup>+</sup> TILs in NSCLC (Gueguen et al., 2021)

# Cluster Plots in LUSC

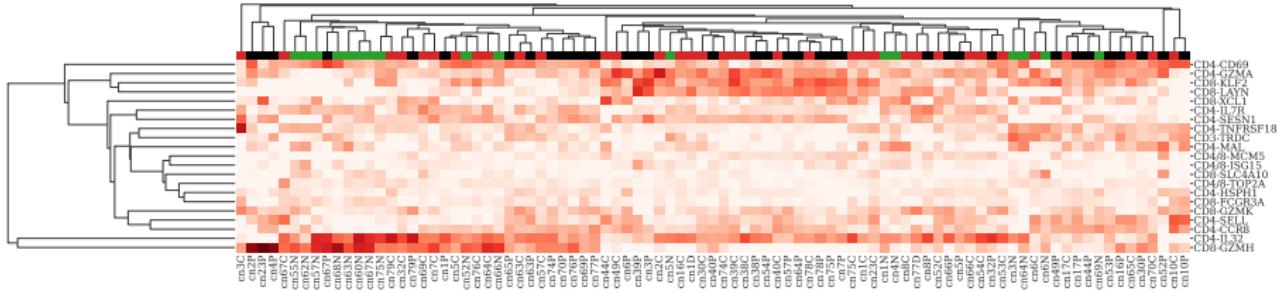
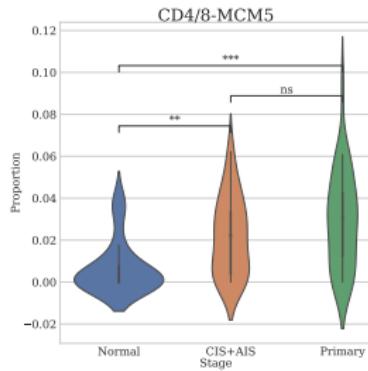
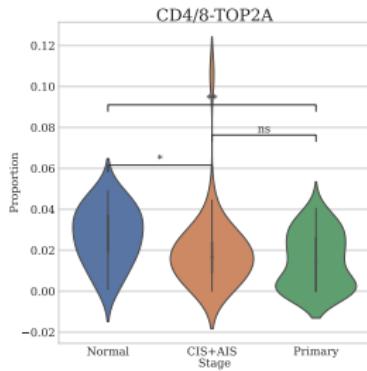


Figure: Cluster Plot in LUAD

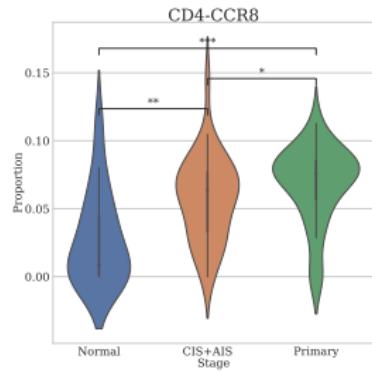
# Violin Plots in LUSC I



(a) CD4/8-MCM5



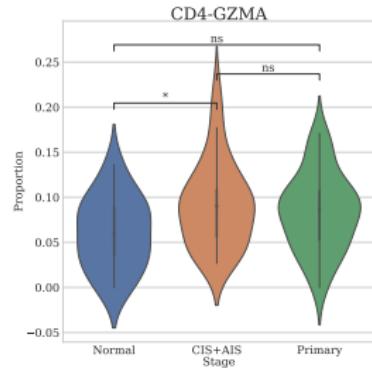
(b) CD4/8-TOP2A



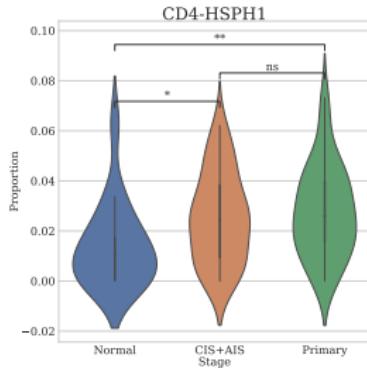
(c) CD4-CCR8

Figure: Violin Plots in LUSC

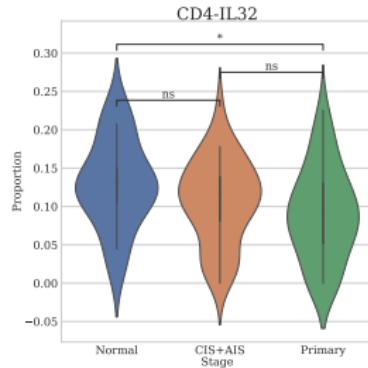
# Violin Plots in LUSC II



(d) CD4-GZMA



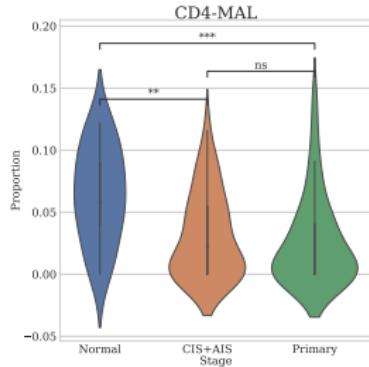
(e) CD4-HSPH1



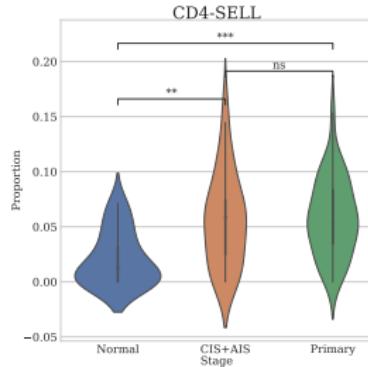
(f) CD4-IL32

Figure: Violin Plots in LUSC

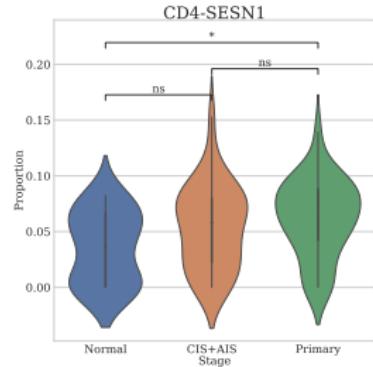
# Violin Plots in LUSC III



(g) CD4-MAL



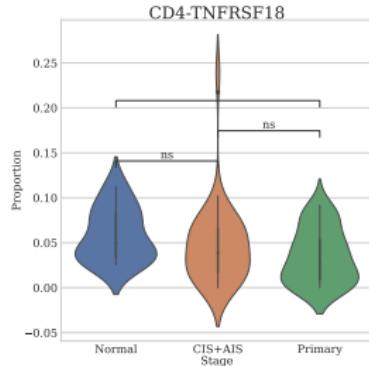
(h) CD4-SELL



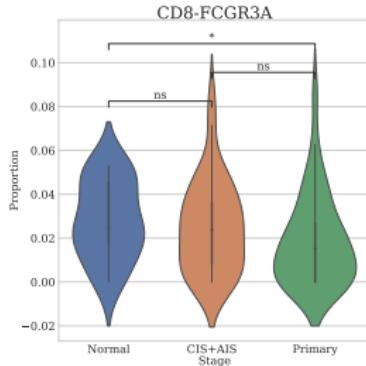
(i) CD4-SESN1

Figure: Violin Plots in LUSC

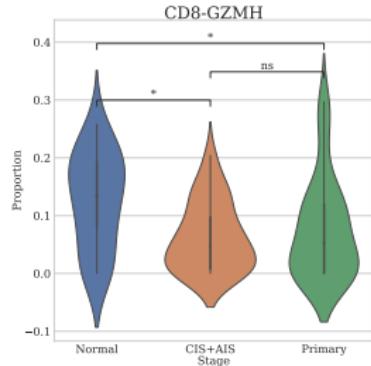
# Violin Plots in LUSC IV



(g) CD4-TNFRSF18



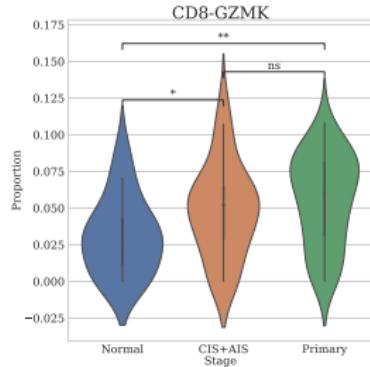
(h) CD8-FCGR3A



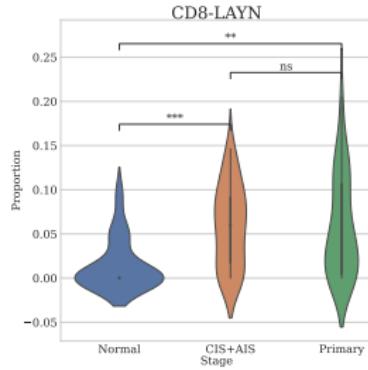
(i) CD8-GZMH

Figure: Violin Plots in LUSC

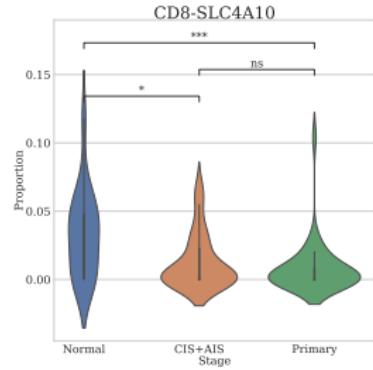
# Violin Plots in LUSC V



(g) CD8-GZMK



(h) CD8-LAYN



(i) CD8-SLC4A10

Figure: Violin Plots in LUSC

# Findings in Bulk Cell Deconvolution with LUSC I

CD4/8-MCM5

content...

CD4/8-TOP2A

content...

CD4-CCR8

content...

CD4-GZMA

content...

CD4-HSPH1

content...

# Findings in Bulk Cell Deconvolution with LUSC II

CD4-IL32

content...

CD4-MAL

content...

CD4-SELL

content...

CD4-SESN1

content...

CD4-TNFRSF18

content...

# Findings in Bulk Cell Deconvolution with LUSC III

CD8-FCGR3A

content...

CD8-GZMH

content...

CD8-GZMK

content...

CD8-LAYN

content...

CD8-SLC4A10

content...

# Cluster Plots in LUAD

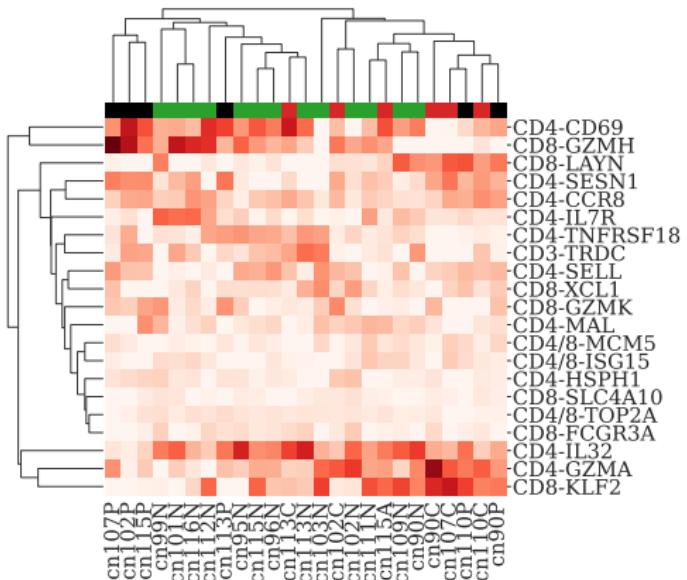
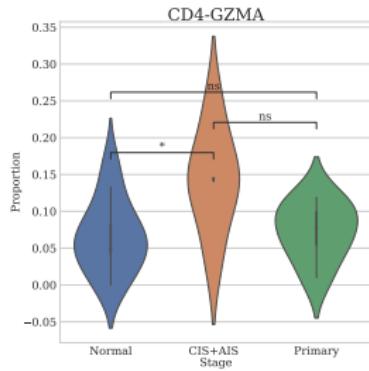
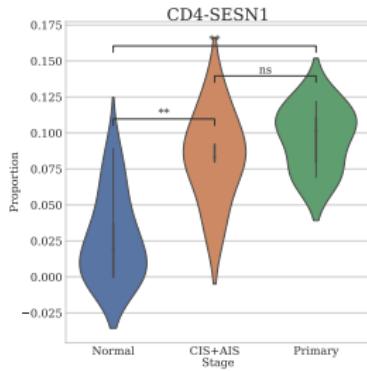


Figure: Cluster Plot in LUAD

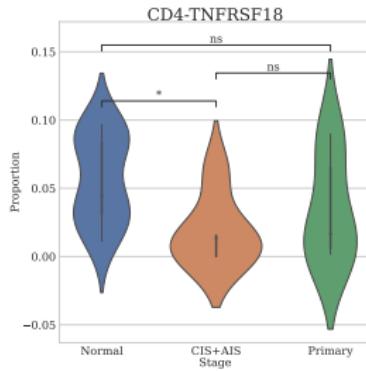
# Violin Plots in LUAD



(a) CD4-GZMA



(b) CD4-SESN1



(c) CD4-TNFRSF18

Figure: Violin Plots in LUAD

# Findings in Bulk Cell Deconvolution with LUAD I

CD4-GZM

content...

CD4-SESN1

content...

CD4-TNFRSF18

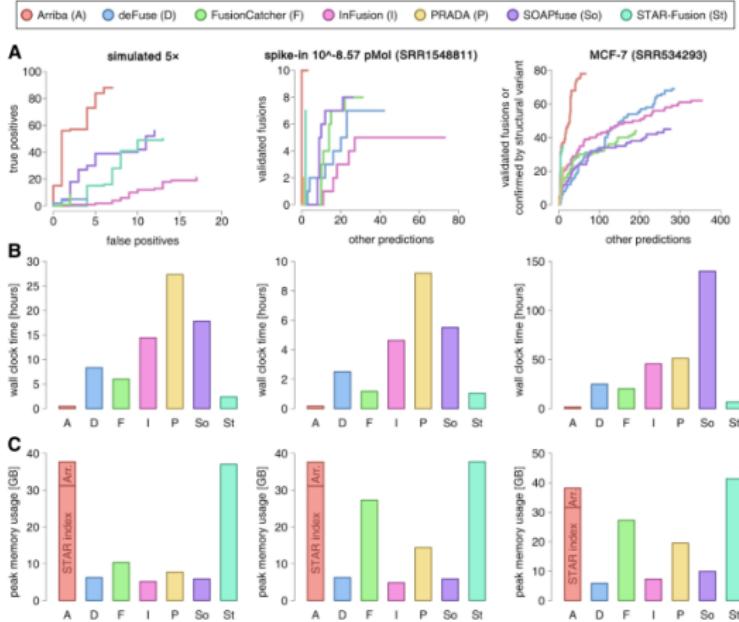
content...

# Findings in Bulk Cell Deconvolution

## 4. Results

### 4.9. Discovery of Gene Fusion

# Arriba?



**Figure:** Benchmark of Arriba versus alternative methods (Uhrig et al., 2021)

# Findings in Gene Fusion Discovery

## 4. Results

### 4.10. Discovery of Mutational Signature

# Mutation Signature?

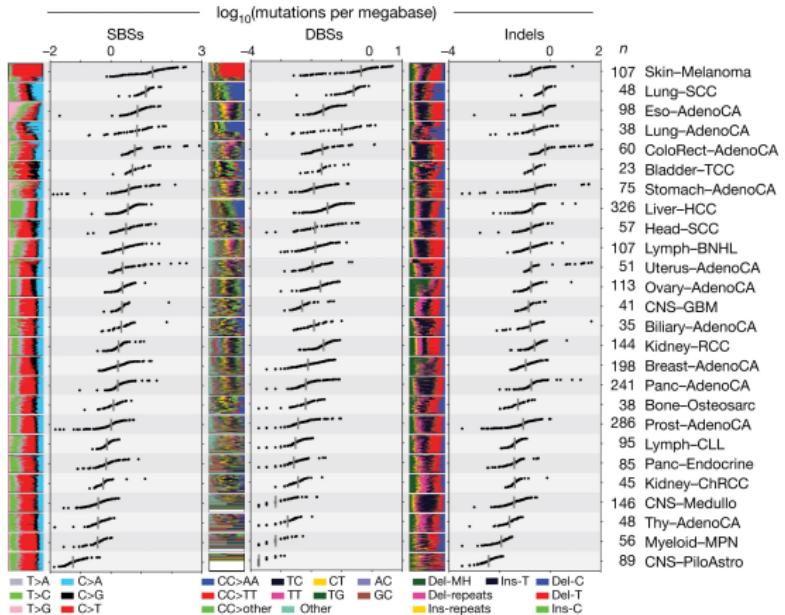
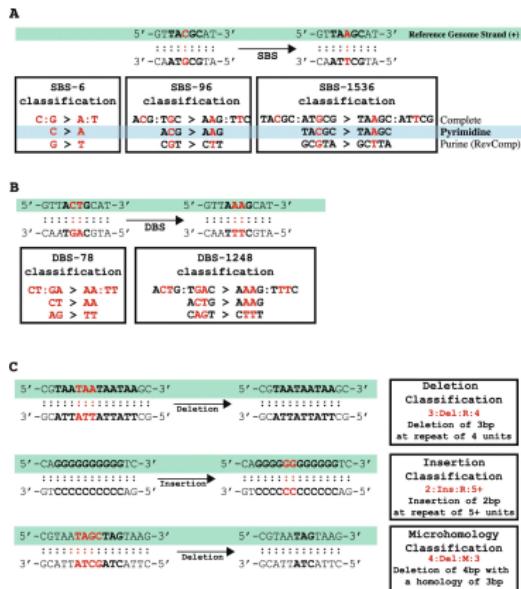


Figure: Mutation Burdens across PCAWG tumor types (Alexandrov et al., 2020)

# SigProfiler?



**Figure:** Classification of mutation signatures by SigProfiler (Bergstrom et al., 2019; Islam et al., 2021; Bergstrom et al., 2020)

## 4. Results

### 4.10. Discovery of Mutational Signature

#### 4.10.1. Single Base Substitutions (SBS)

# SBS Signatures I

## SBS1

- An endogenous mutational process (Nik-Zainal et al., 2012a)
- generates G>T mismatches in double-stranded DNA
- Failure ↓ to detect & remove these mismatches

## SBS2

- Activity of the AID/APOBEC family of cytidine deaminases (Nik-Zainal et al., 2012a)
  - ① APOBEC3A is probably responsible in human cancer
  - ② APOBEC3B may also contribute
- may be generated directly by DNA replication

## SBS4

- Tobacco smoking (Alexandrov et al., 2013)
- Exposed to tobacco carcinogens e.g. benzopyrene

## SBS5

- Unknown (Alexandrov et al., 2013)
- SBS5 ↑ in bladder cancer
- SBS5 ↑ in many cancer types ∵ Tobacco smoking

## SBS10b

- Polymerase ε exonuclease domain mutations (Alexandrov et al., 2020)

# SBS Signatures III

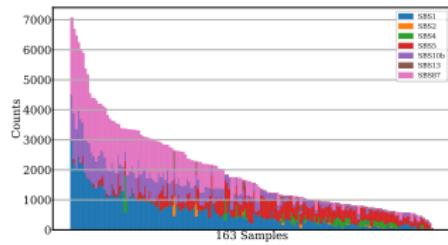
## SBS13

- Activity of the AID/APOBEC family of cytidine deaminases (Nik-Zainal et al., 2012b)
- SBS13 is usually found with SBS2

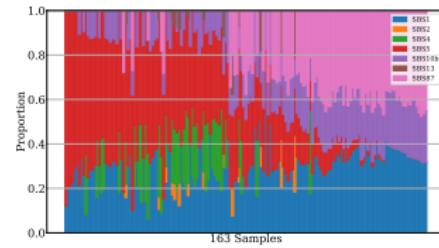
## SBS87

- Thiopurine chemotherapy treatment (Li et al., 2020)

# SBS in LUSC I



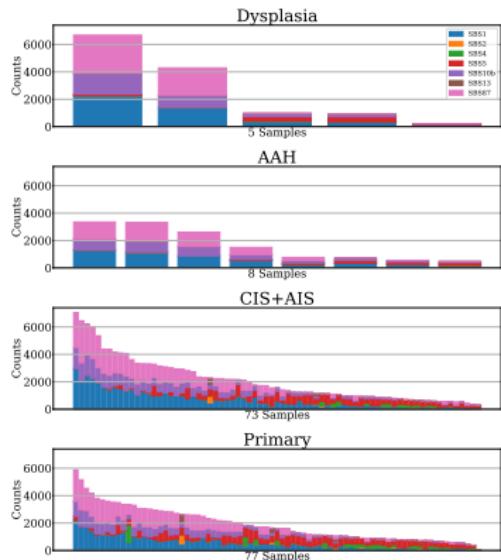
(a) Absolute



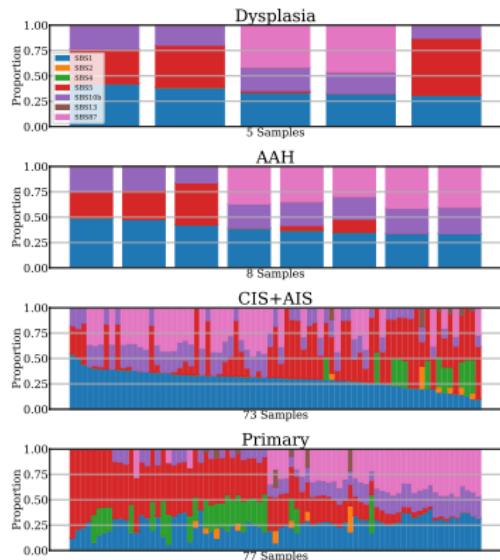
(b) Relative

Figure: SBS Bar Plot in LUSC

# SBS in LUSC II



(a) Absolute



(b) Relative

Figure: SBS Bar Plot by Cancer Subtype in LUSC

# SBS in LUSC with Smoking I

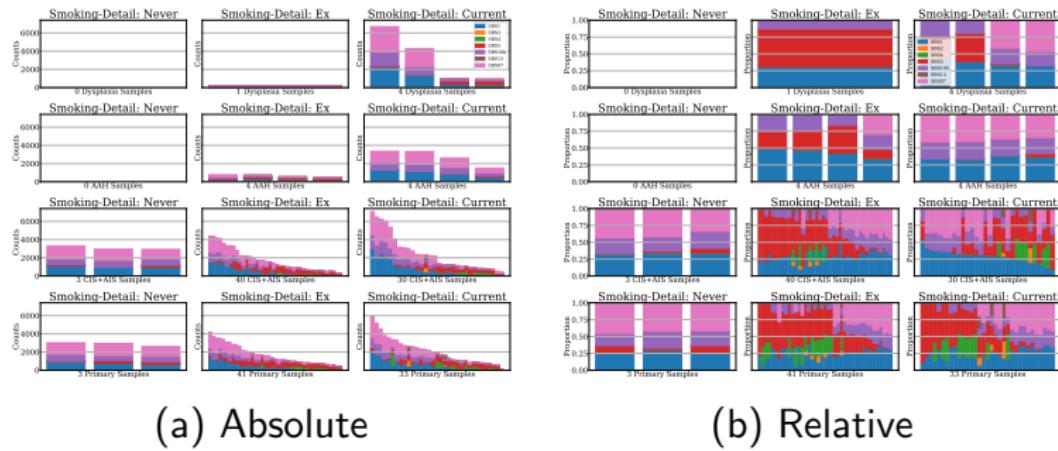
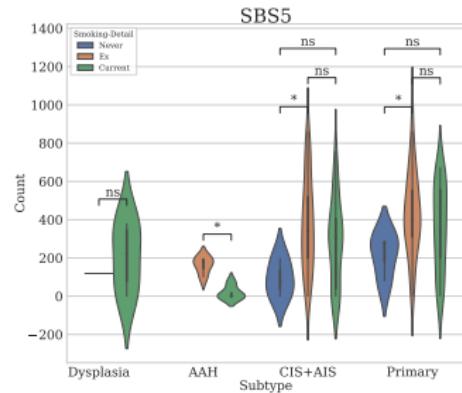
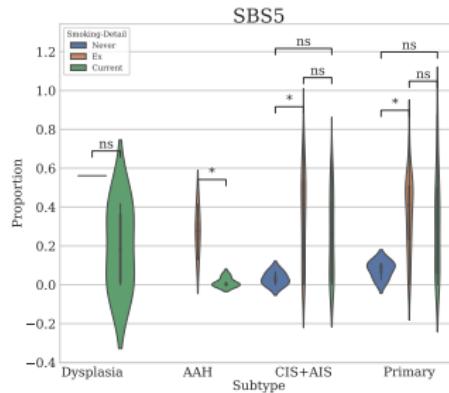


Figure: SBS Bar Plot by Cancer Subtype & Smoking in LUSC

# SBS in LUSC with Smoking II



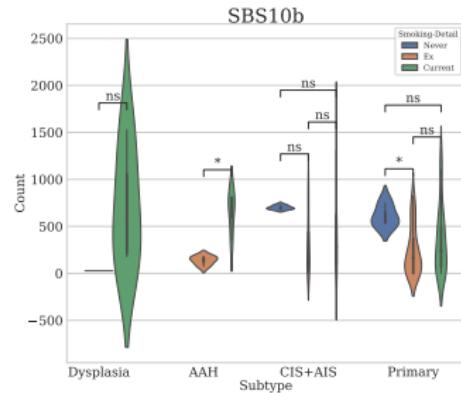
(a) Absolute



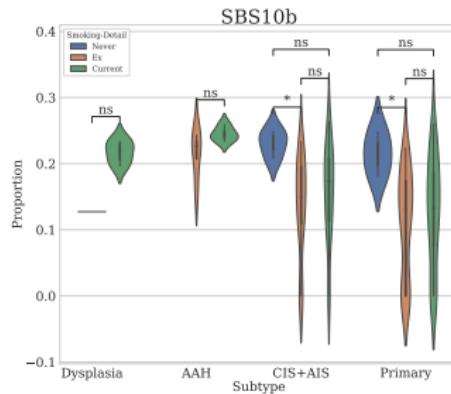
(b) Relative

**Figure:** SBS5 Signature in LUSC with Smoking

# SBS in LUSC with Smoking III



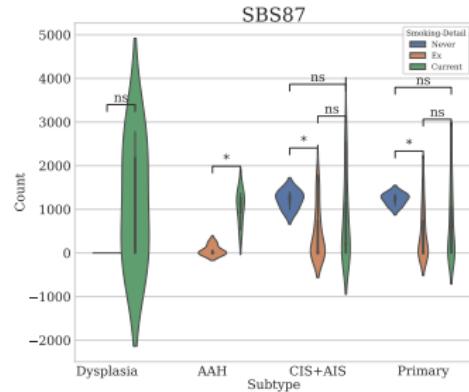
(a) Absolute



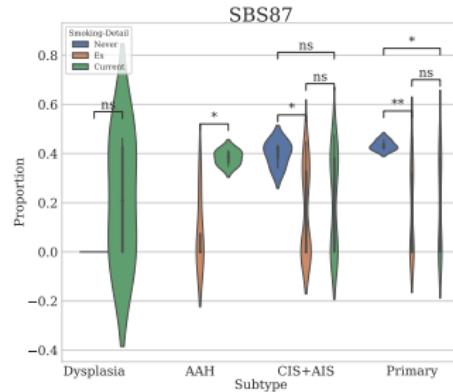
(b) Relative

Figure: SBS10b Signature in LUSC with Smoking

# SBS in LUSC with Smoking IV



(a) Absolute



(b) Relative

Figure: SBS87 Signature in LUSC with Smoking

# SBS in LUSC with Recurrence I

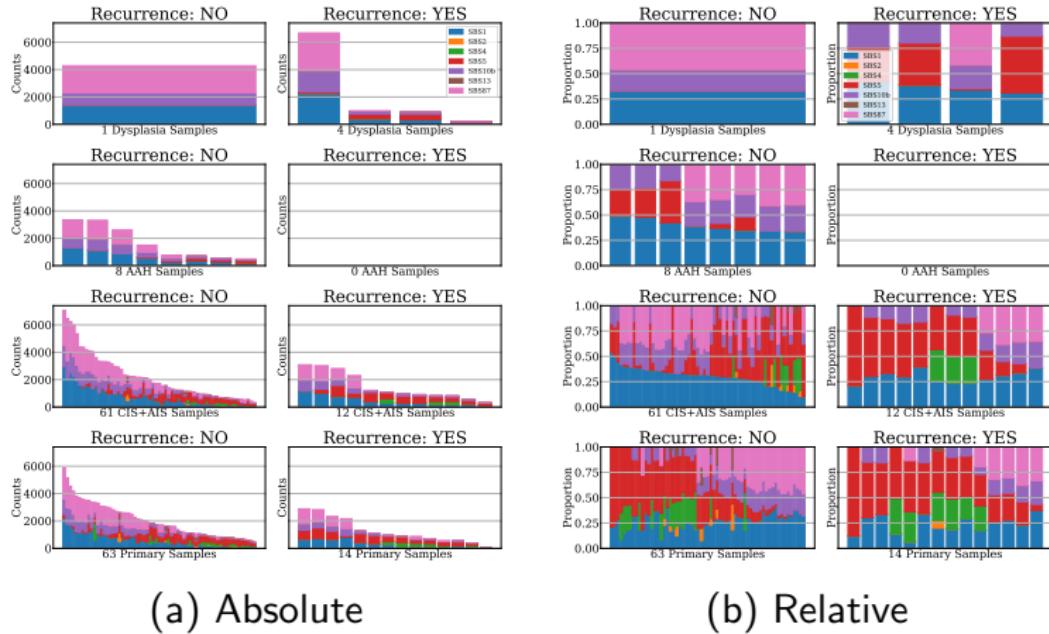
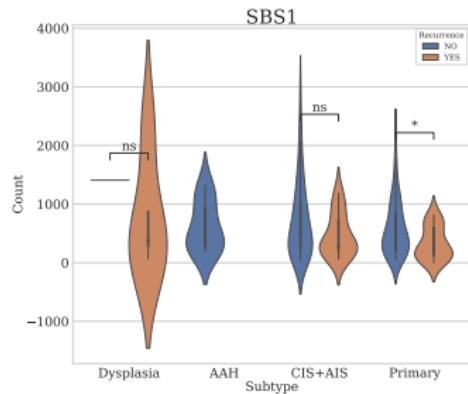
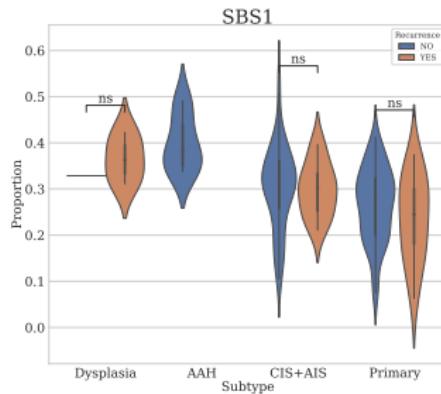


Figure: SBS Bar Plot by Cancer Subtype & Recurrence in LUSC

# SBS in LUSC with Recurrence II



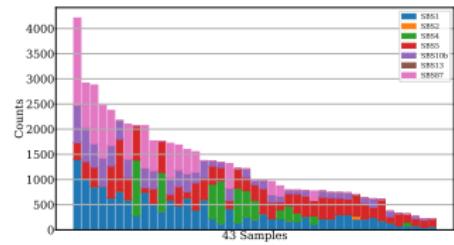
(a) Absolute



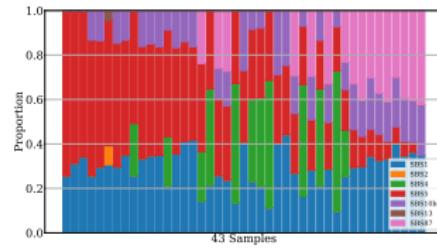
(b) Relative

**Figure:** SBS1 Signature in LUSC with Recurrence

# SBS in LUAD I



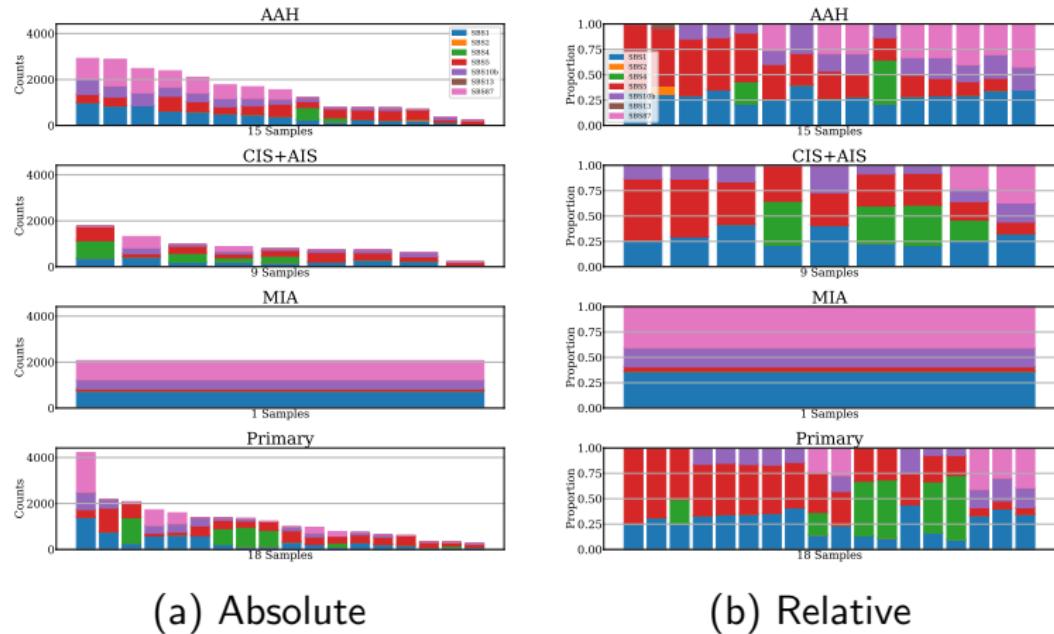
(a) Absolute



(b) Relative

Figure: SBS Bar Plot in LUSC

# SBS in LUAD II



(a) Absolute

(b) Relative

Figure: SBS Bar Plot by Cancer Subtype in LUSC

# SBS in LUAD with Smoking I

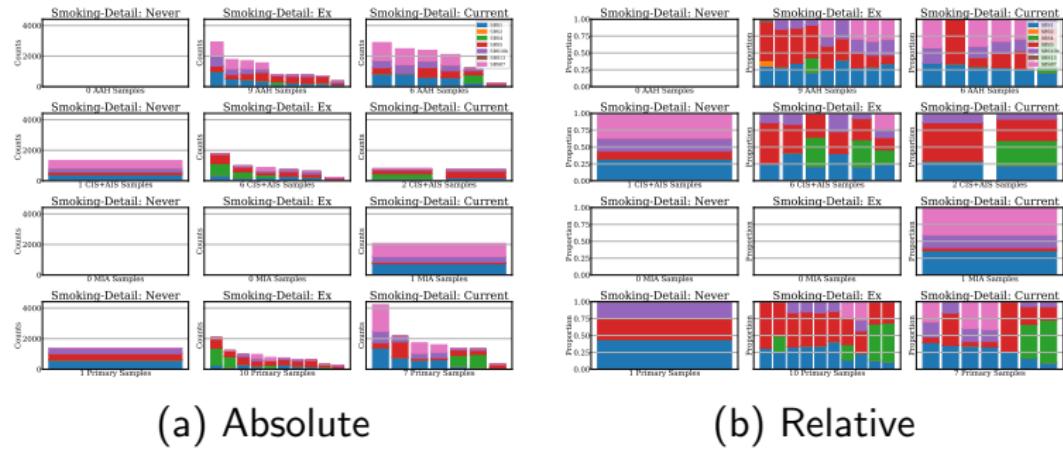
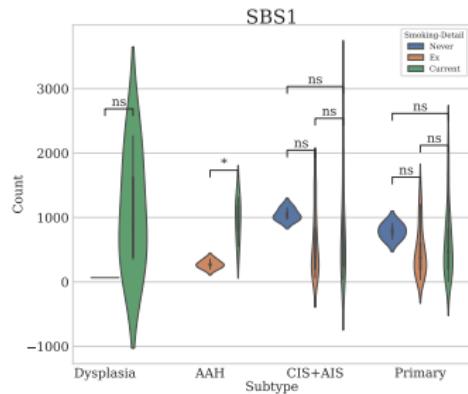
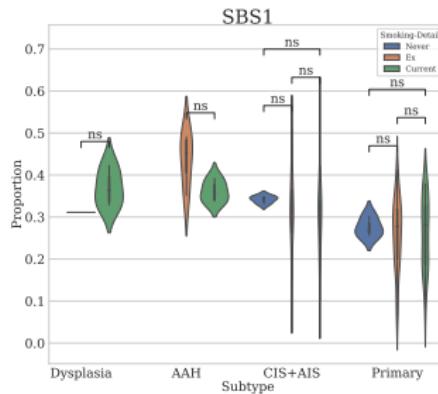


Figure: SBS Bar Plot by Cancer Subtype & Smoking in LUAD

# SBS in LUAD with Smoking II



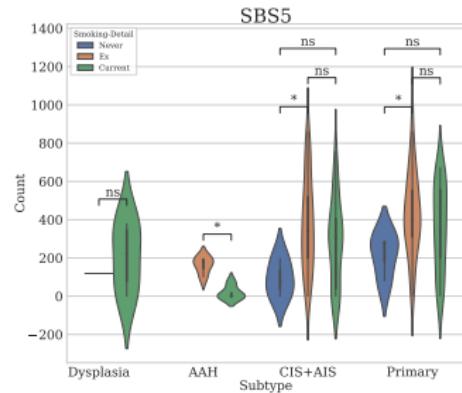
(a) Absolute



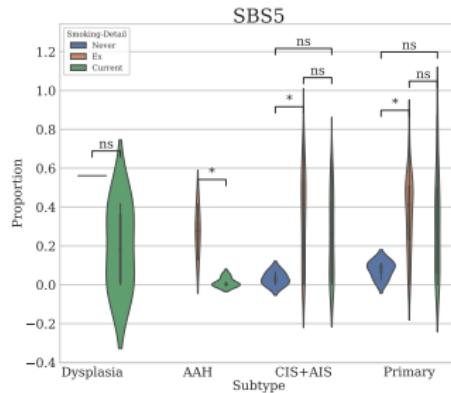
(b) Relative

**Figure:** SBS1 Signature in LUAD with Smoking

# SBS in LUAD with Smoking III



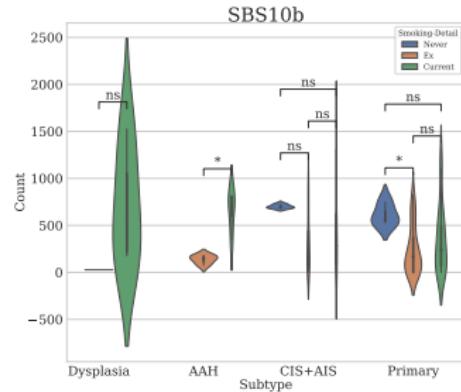
(a) Absolute



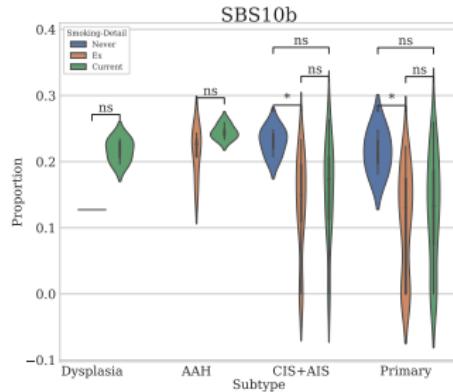
(b) Relative

**Figure:** SBS5 Signature in LUAD with Smoking

# SBS in LUAD with Smoking IV



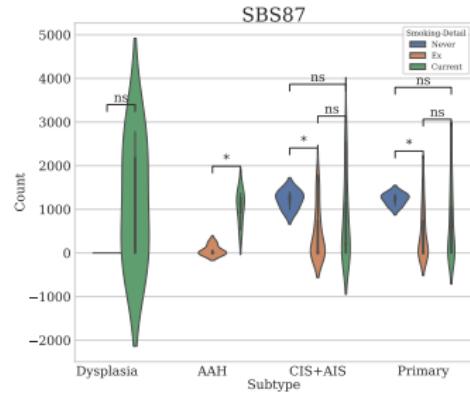
(a) Absolute



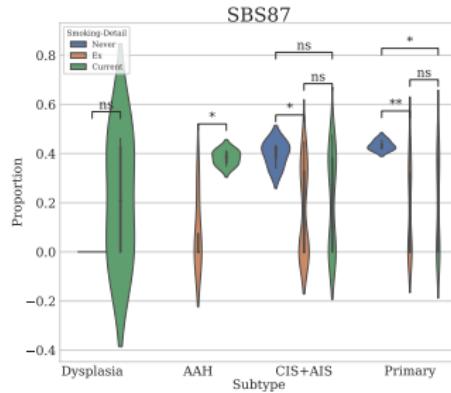
(b) Relative

Figure: SBS10b Signature in LUAD with Smoking

# SBS in LUAD with Smoking V



(a) Absolute



(b) Relative

Figure: SBS87 Signature in LUAD with Smoking

# SBS in LUAD with Recurrence I

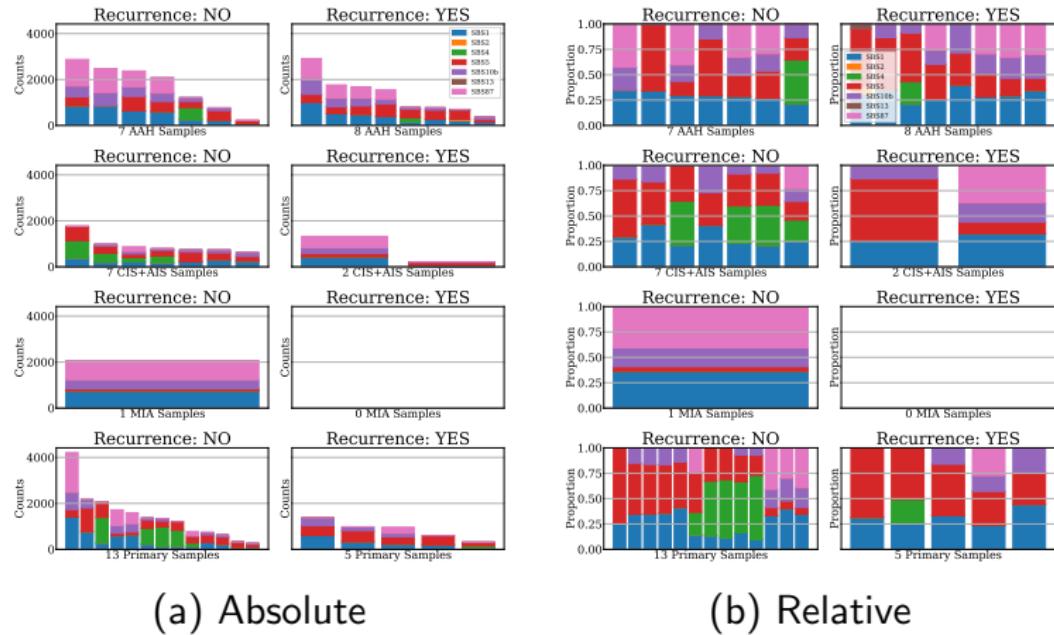
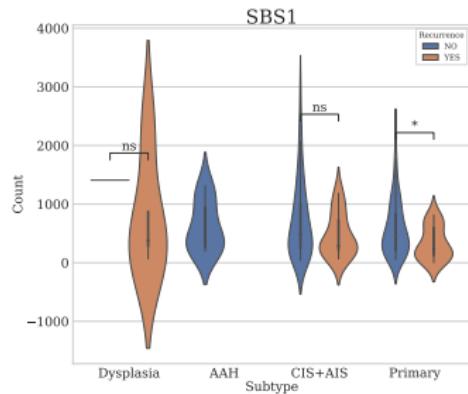
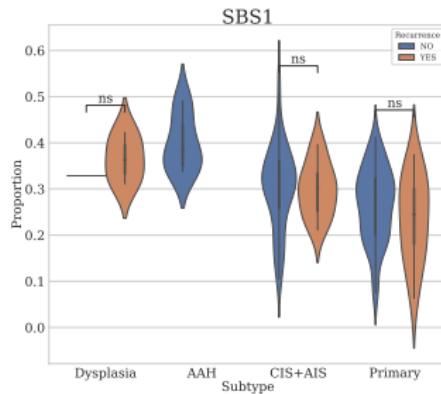


Figure: SBS Bar Plot by Cancer Subtype & Recurrence in LUAD

# SBS in LUAD with Recurrence II



(a) Absolute



(b) Relative

**Figure:** SBS1 Signature in LUAD with Recurrence

## 4. Results

### 4.10. Discovery of Mutational Signature

#### 4.10.2. Double Base Substitutions (DBS)

# DBS Signatures I

## DBS2

- Tobacco smoking (J.-M. Chen, Férec, & Cooper, 2013)
- Other endogenous/exogenous mutagens e.g. acetaldehyde

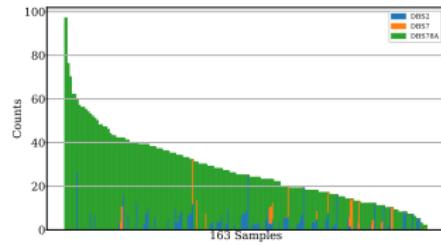
## DBS7

- Defective ↓ DNA mismatch repair (Alexandrov et al., 2020)

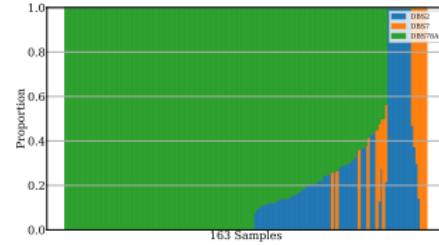
## DBS78A

content...

# DBS in LUSC I



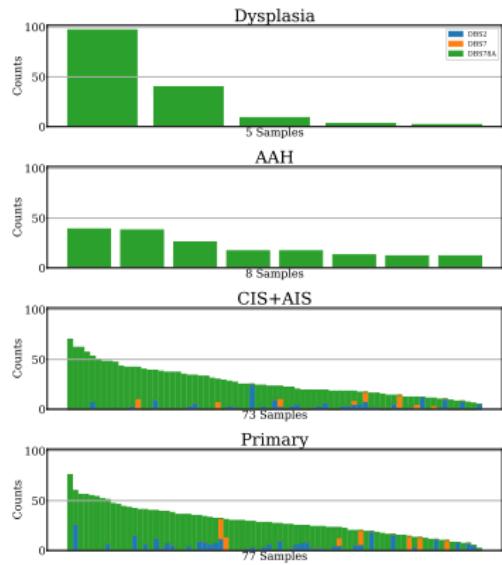
(a) Absolute



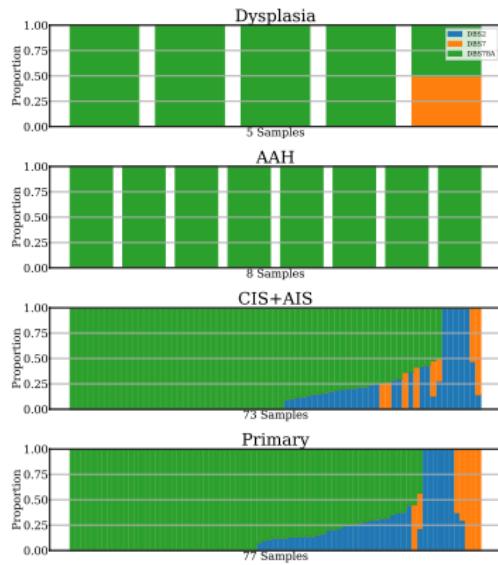
(b) Relative

Figure: DBS Bar Plot in LUSC

# DBS in LUSC II



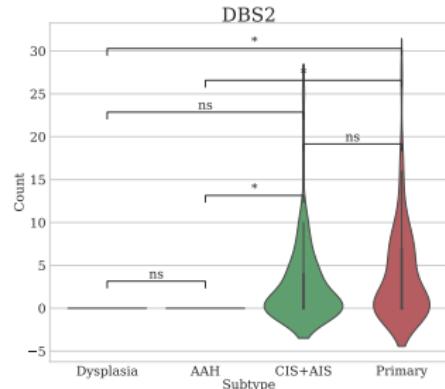
(a) Absolute



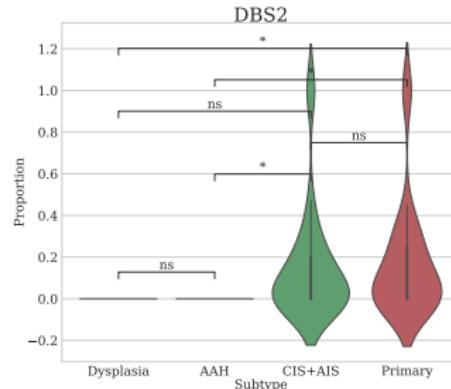
(b) Relative

Figure: DBS Bar Plot by Cancer Subtype in LUSC

# DBS in LUSC III



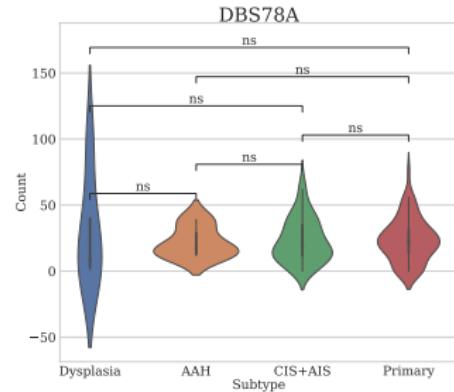
(a) Absolute



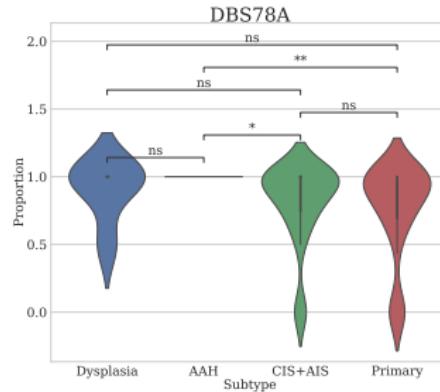
(b) Relative

Figure: DBS2 Signature in LUSC

# DBS in LUSC IV



(a) Absolute



(b) Relative

Figure: DBS78A Signature in LUSC

# DBS in LUSC with Smoking I

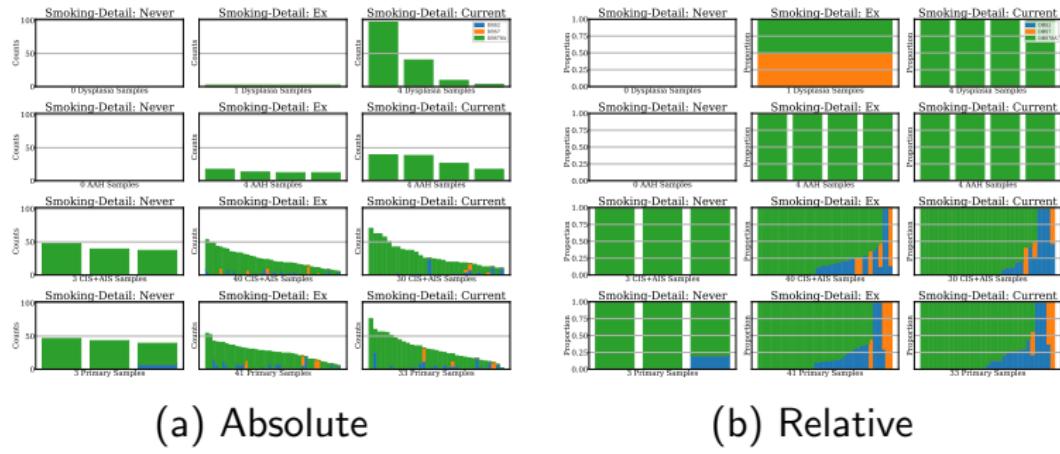
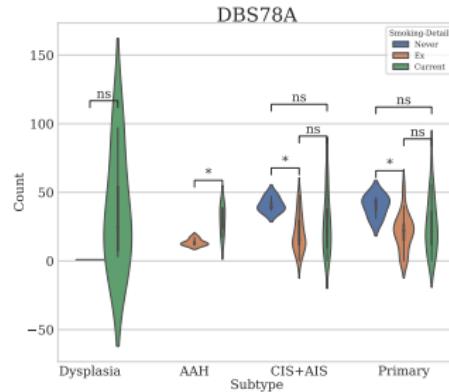
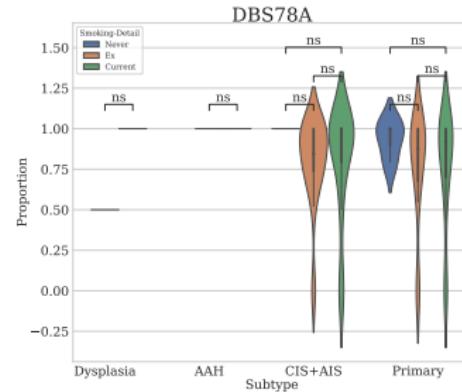


Figure: DBS Bar Plot by Cancer Subtype & Smoking in LUSC

# DBS in LUSC with Smoking II



(a) Absolute



(b) Relative

Figure: DBS78A Signature in LUSC with Smoking

# DBS in LUSC with Recurrence

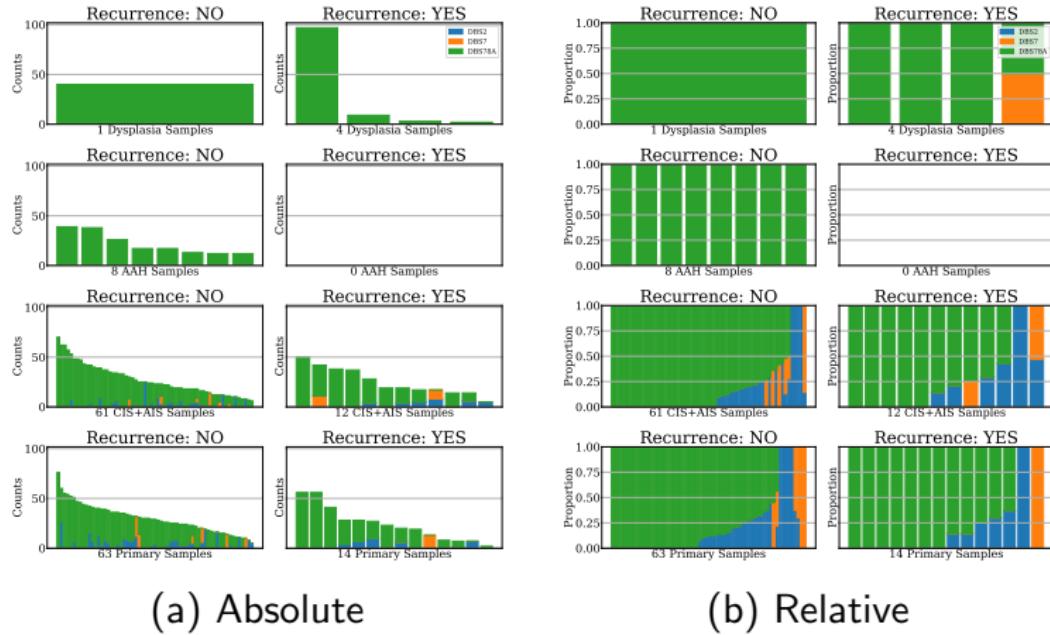
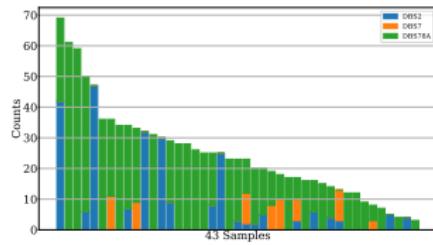
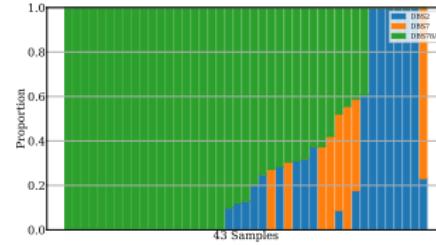


Figure: DBS Bar Plot by Cancer Subtype & Recurrence in LUSC

# DBS in LUAD I



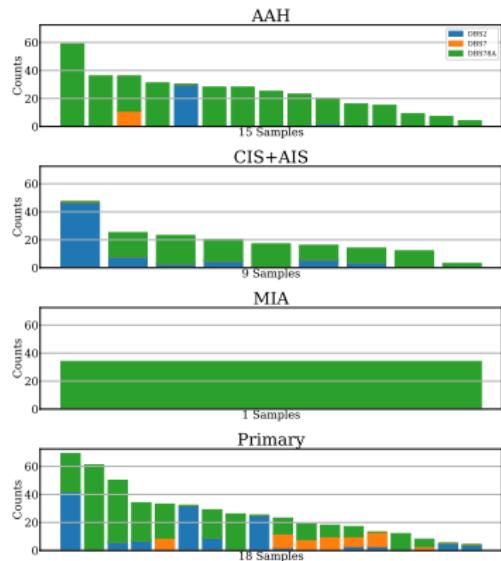
(a) Absolute



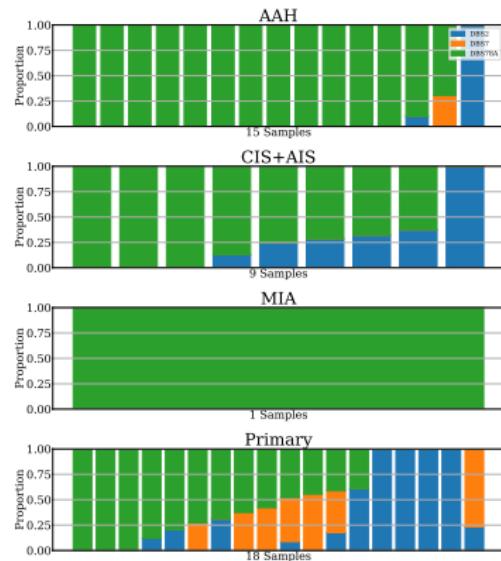
(b) Relative

Figure: DBS Bar Plot in LUAD

# DBS in LUAD II



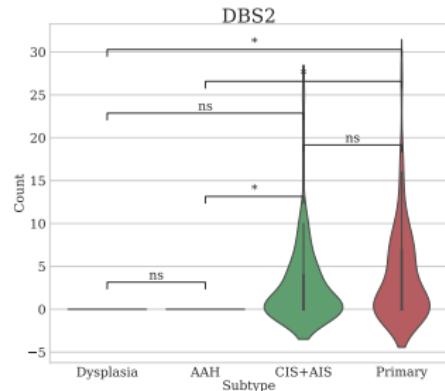
(a) Absolute



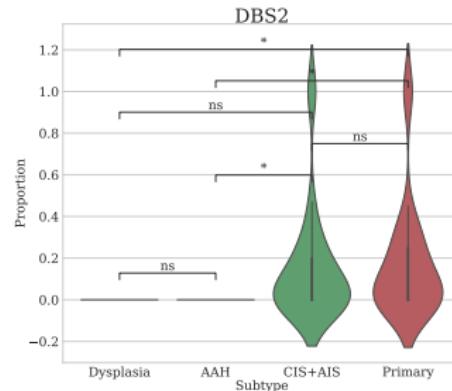
(b) Relative

Figure: DBS Bar Plot by Cancer Subtype in LUAD

# DBS in LUAD III



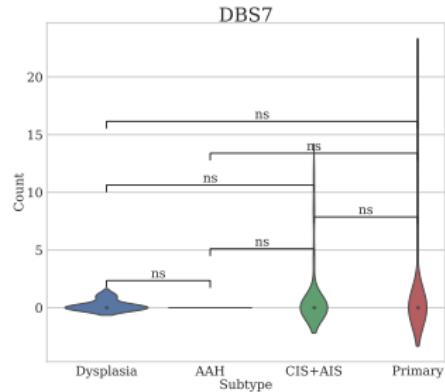
(a) Absolute



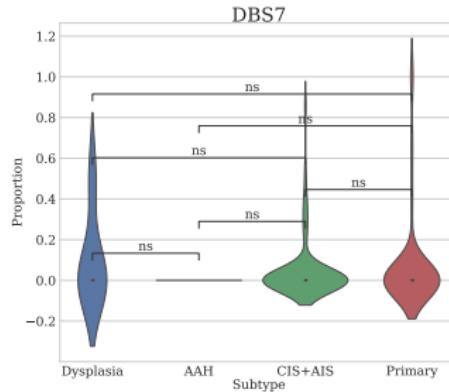
(b) Relative

Figure: DBS2 Signature in LUSC

# DBS in LUAD IV



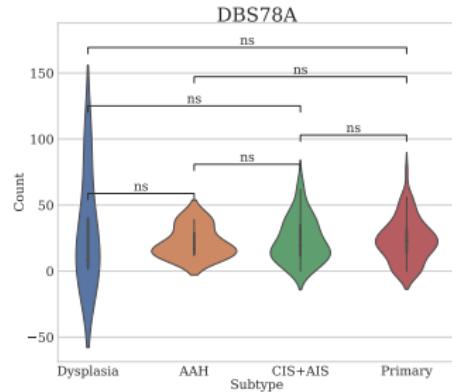
(a) Absolute



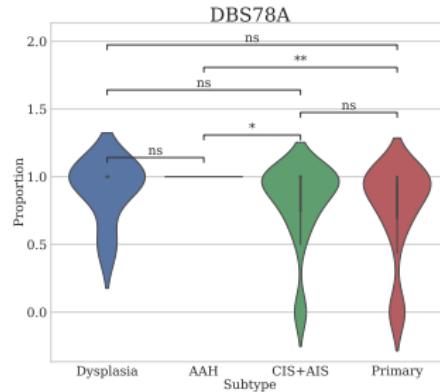
(b) Relative

Figure: DBS7 Signature in LUSC

# DBS in LUAD V



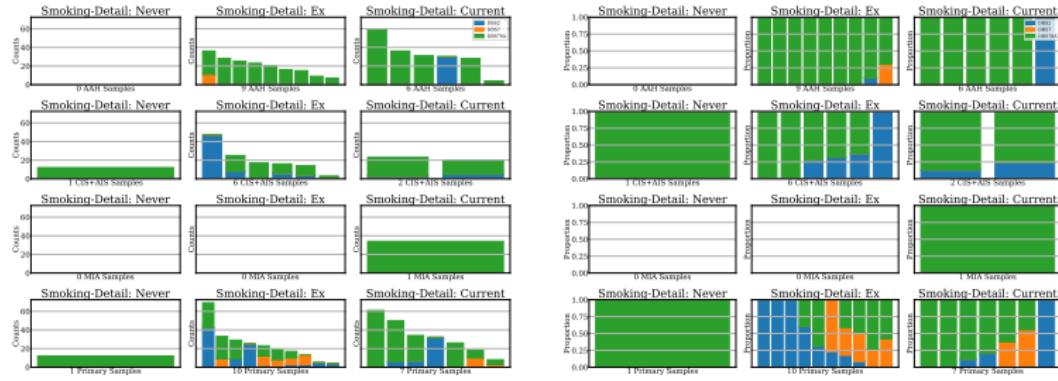
(a) Absolute



(b) Relative

Figure: DBS78A Signature in LUSC

# DBS in LUAD with Smoking I

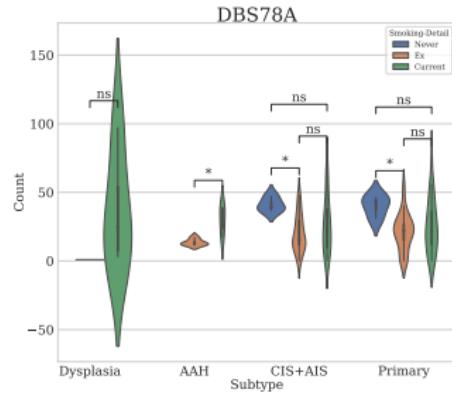


(a) Absolute

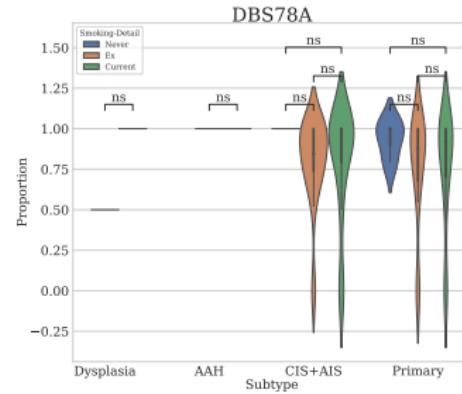
(b) Relative

Figure: DBS Bar Plot by Cancer Subtype & Smoking in LUAD

# DBS in LUAD with Smoking II



(a) Absolute



(b) Relative

Figure: DBS78A Signature in LUSC in Smoking

# DBS in LUAD with Recurrence

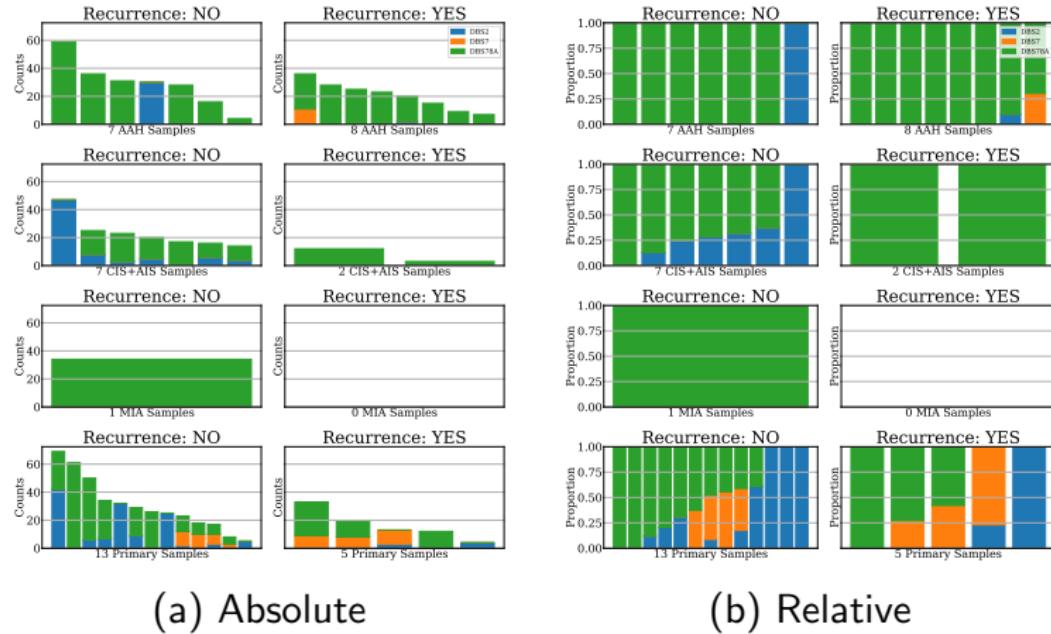


Figure: DBS Bar Plot by Cancer Subtype & Recurrence in LUAD

## 4. Results

### 4.10. Discovery of Mutational Signature

#### 4.10.3. Short insertions & Deletions (Indels)

# Indel signatures I

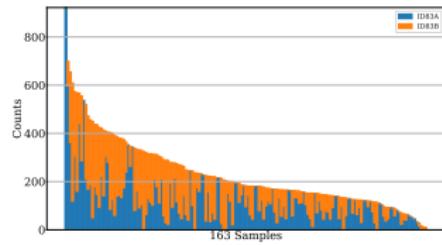
ID83A

content...

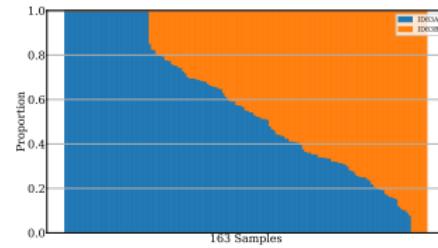
ID83B

content...

# Indels in LUSC I



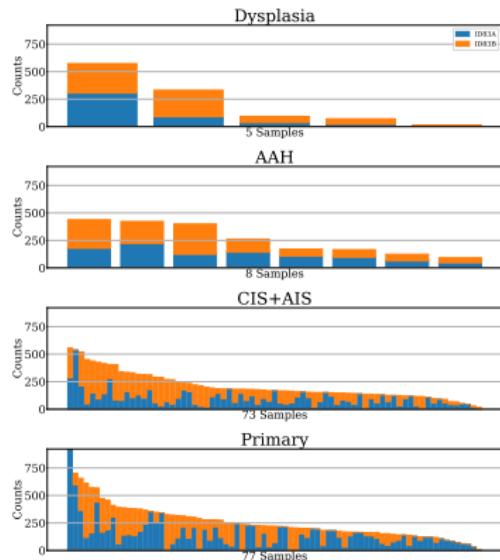
(a) Absolute



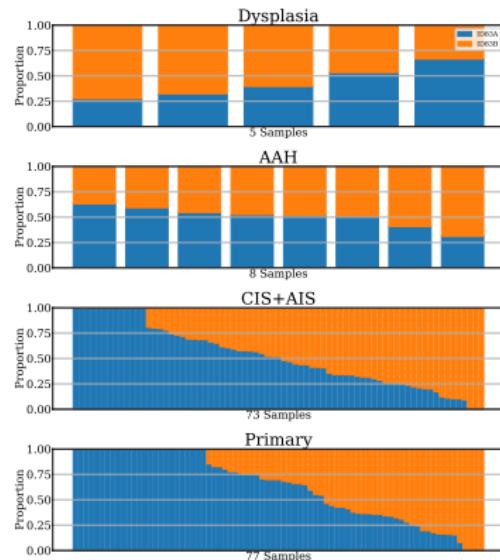
(b) Relative

Figure: Indel Bar Plot in LUSC

# Indels in LUSC II



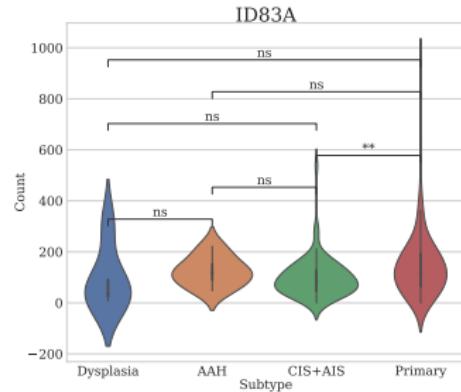
(a) Absolute



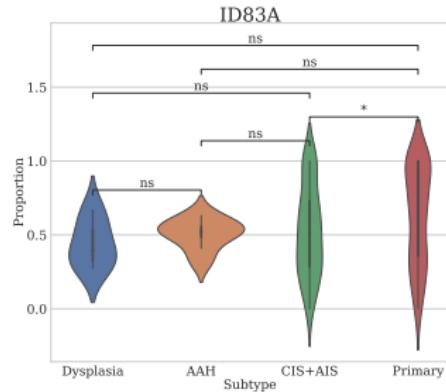
(b) Relative

Figure: Indel Bar Plot by Cancer Subtype in LUSC

# Indels in LUSC III



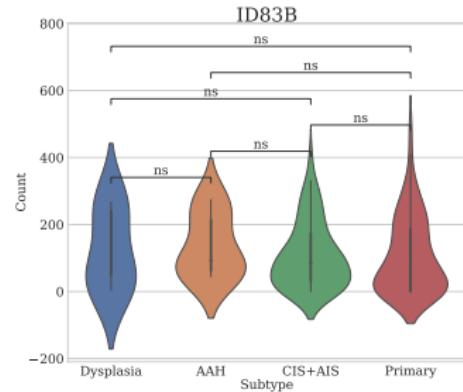
(a) Absolute



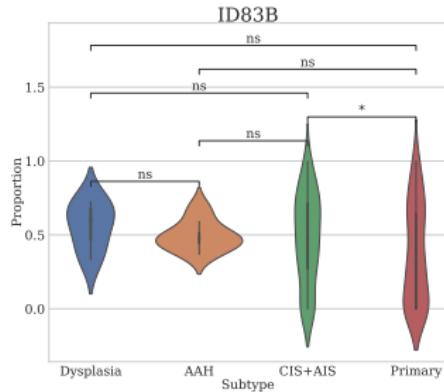
(b) Relative

Figure: Indel83A Signature in LUSC

# Indels in LUSC IV



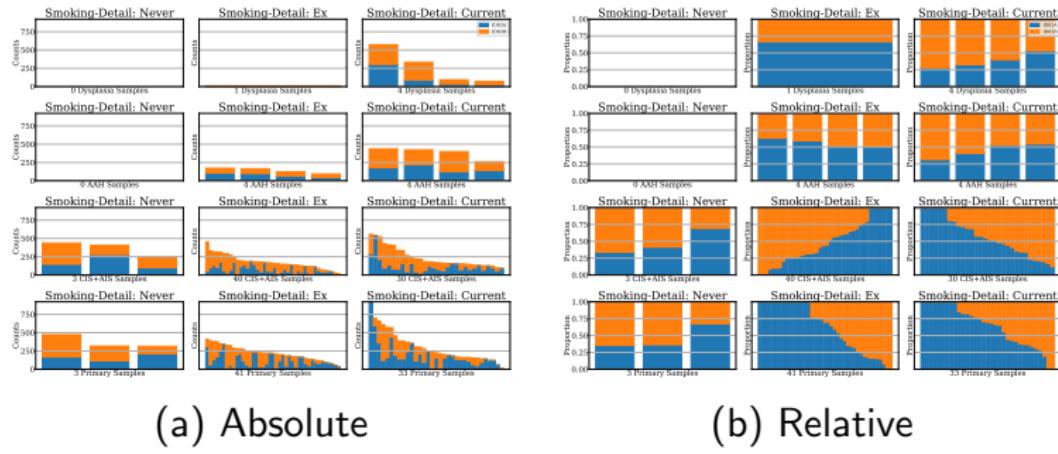
(a) Absolute



(b) Relative

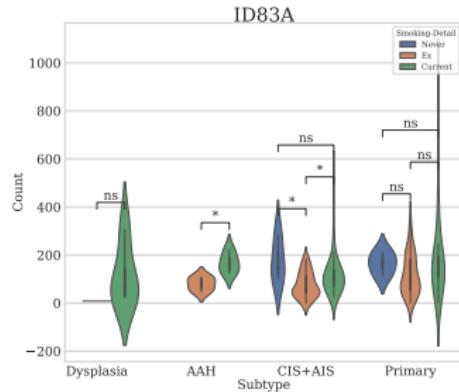
Figure: Indel83B Signature in LUSC

# Indel in LUSC with Smoking I

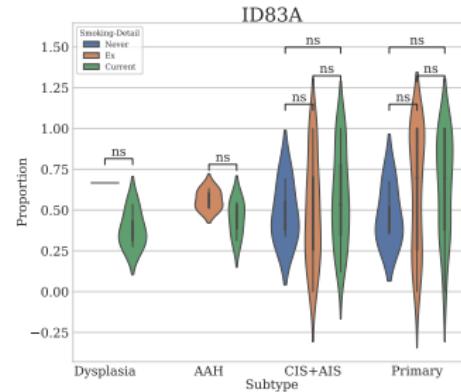


**Figure:** Indel Bar Plot by Cancer Subtype & Smoking in LUSC

# Indel in LUSC with Smoking II



(a) Absolute



(b) Relative

Figure: Indel83A Signature in LUSC with Smoking

# Indel in LUSC with Recurrence

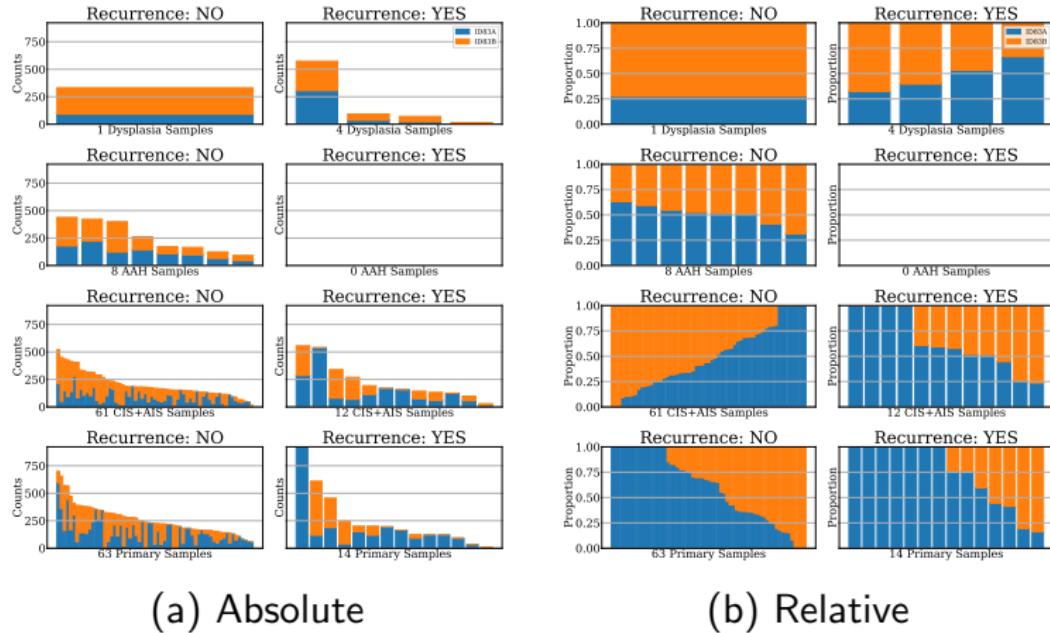
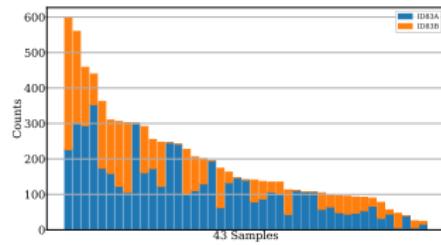
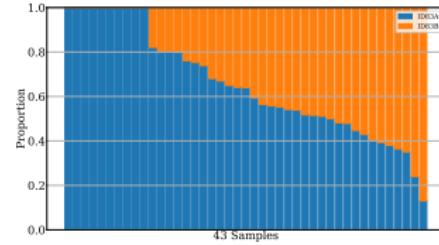


Figure: Indel Bar Plot by Cancer Subtype & Recurrence in LUSC

# Indels in LUAD I



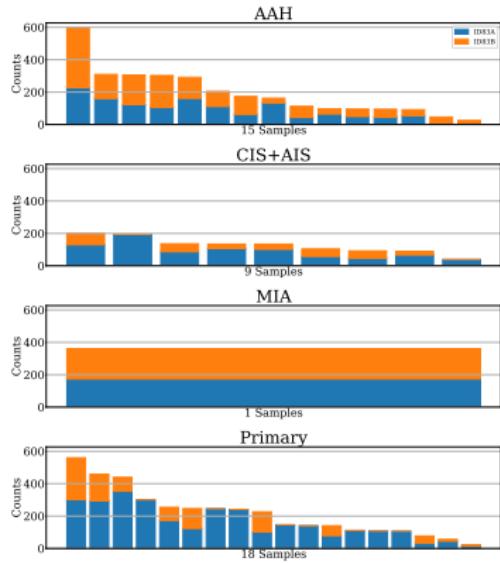
(a) Absolute



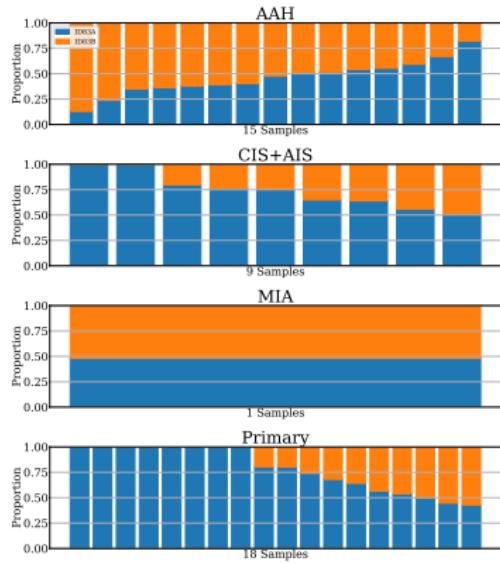
(b) Relative

Figure: Indel Bar Plot in LUAD

# Indels in LUAD II



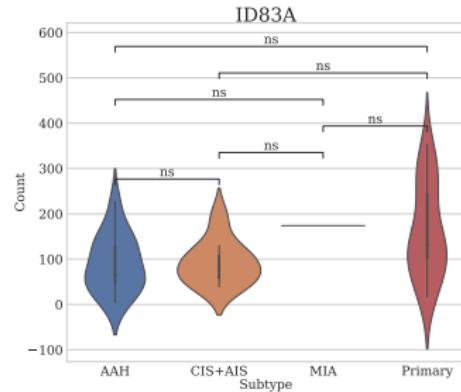
(a) Absolute



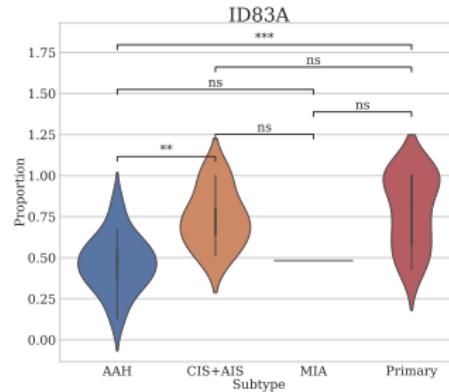
(b) Relative

Figure: Indel Bar Plot by Cancer Subtype in LUAD

# Indels in LUAD III



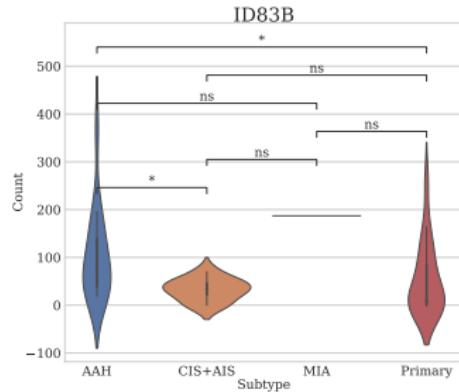
(a) Absolute



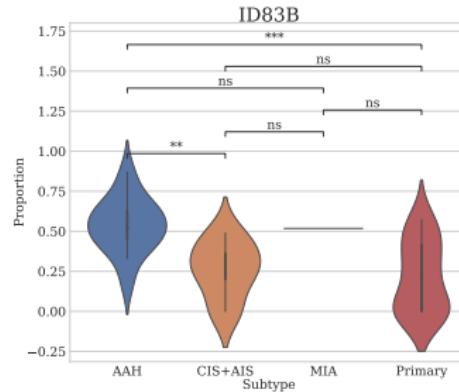
(b) Relative

Figure: Indel83A Signature in LUAD

# Indels in LUAD IV



(a) Absolute



(b) Relative

Figure: Indel83B Signature in LUAD

# Indel in LUAD with Smoking

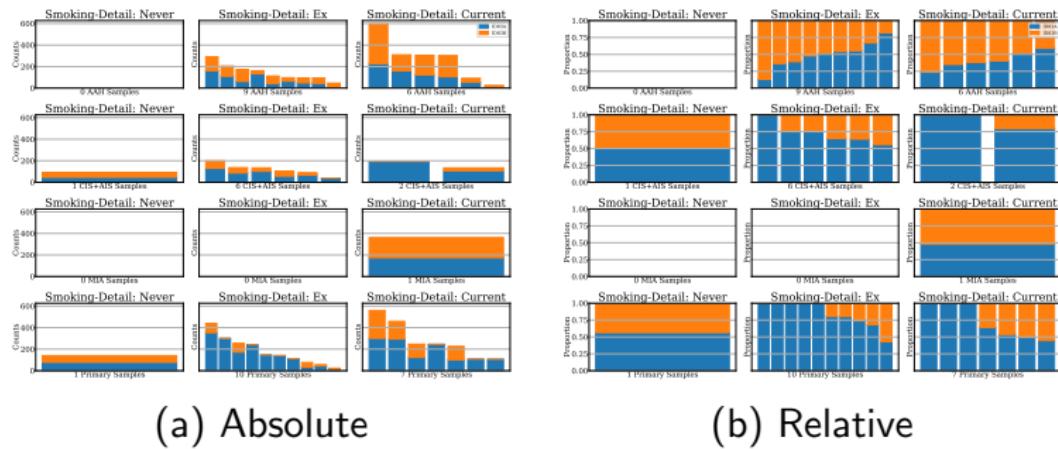
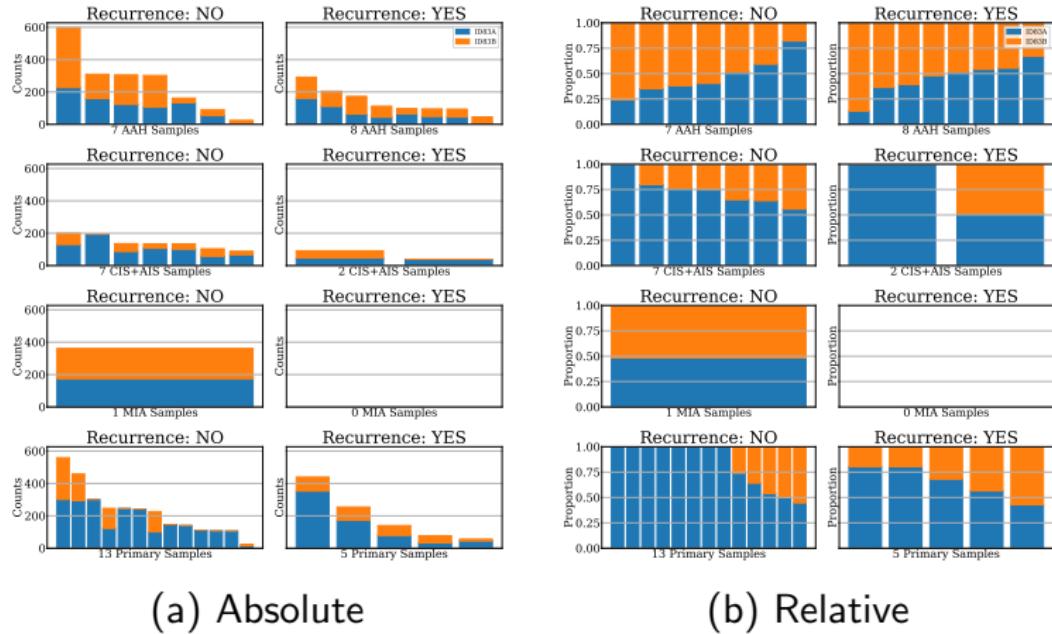


Figure: Indel Bar Plot by Cancer Subtype & Smoking in LUAD

# Indel in LUAD with Recurrence



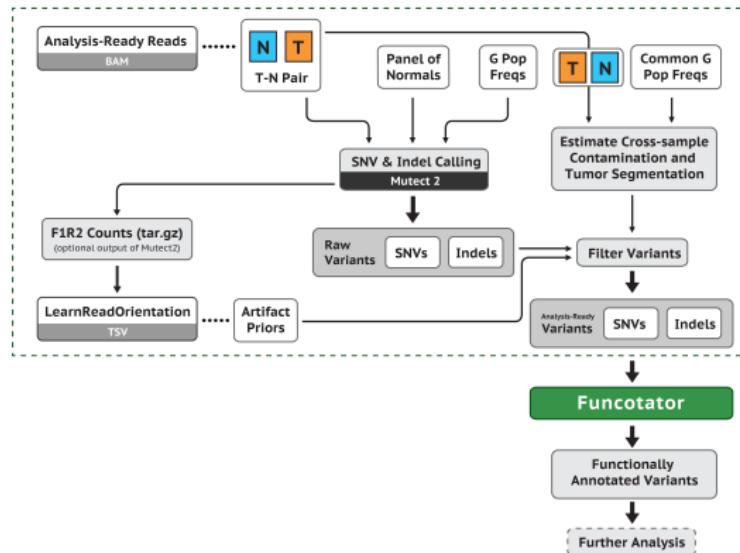
**Figure:** Indel Bar Plot by Cancer Subtype & Recurrence in LUAD

# Findings in Mutation Signature

## 4. Results

### 4.11. Clinical Data with Point Mutation

# Mutect2?



**Figure:** Somatic short variant discovery workflow (Van der Auwera et al., 2013; DePristo et al., 2011)

## 4. Results

### 4.11. Clinical Data with Point Mutation

#### 4.11.1. For Smoking

# LUSC with Smoking

Table: LUSC WES Data with Smoking

Smoking?	Stage	Number of Samples	
		Normal	CIS+AIS
Never	Normal	3	
	CIS+AIS	3	
	Primary	3	
	Total	9	
Ex	Normal	41	
	Dysplasia	1	
	AAH	4	
	CIS+AIS	40	
	Primary	41	
	Total	127	
Current	Normal	33	
	Dysplasia	4	
	AAH	4	
	CIS+AIS	30	
	Primary	33	
	Total	104	

# Clinical Data about LUSC for Smoking I

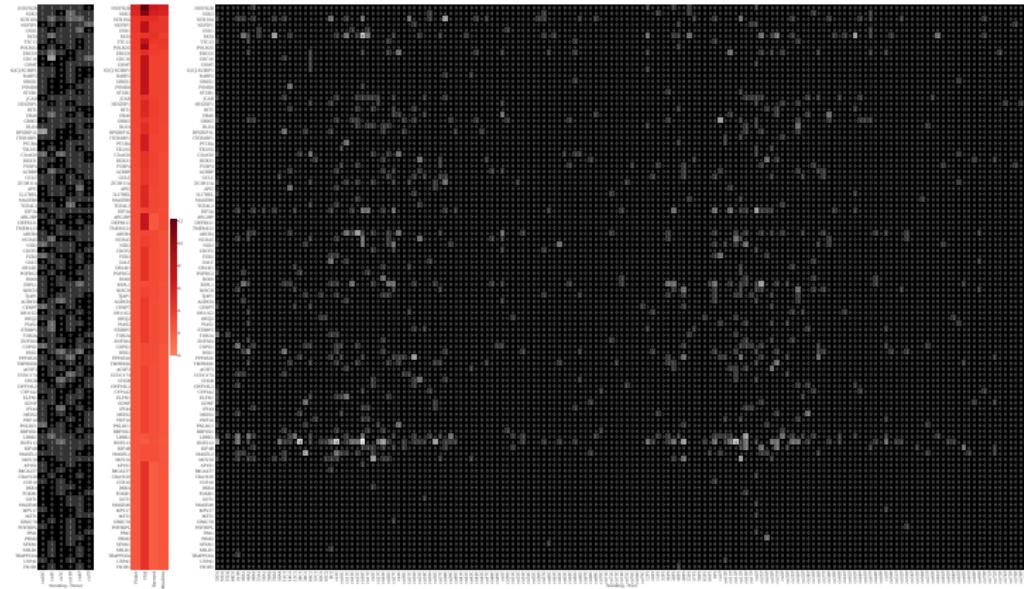


Figure: Clinical Data about LUSC for Smoking

# Clinical Data about LUSC for Smoking II

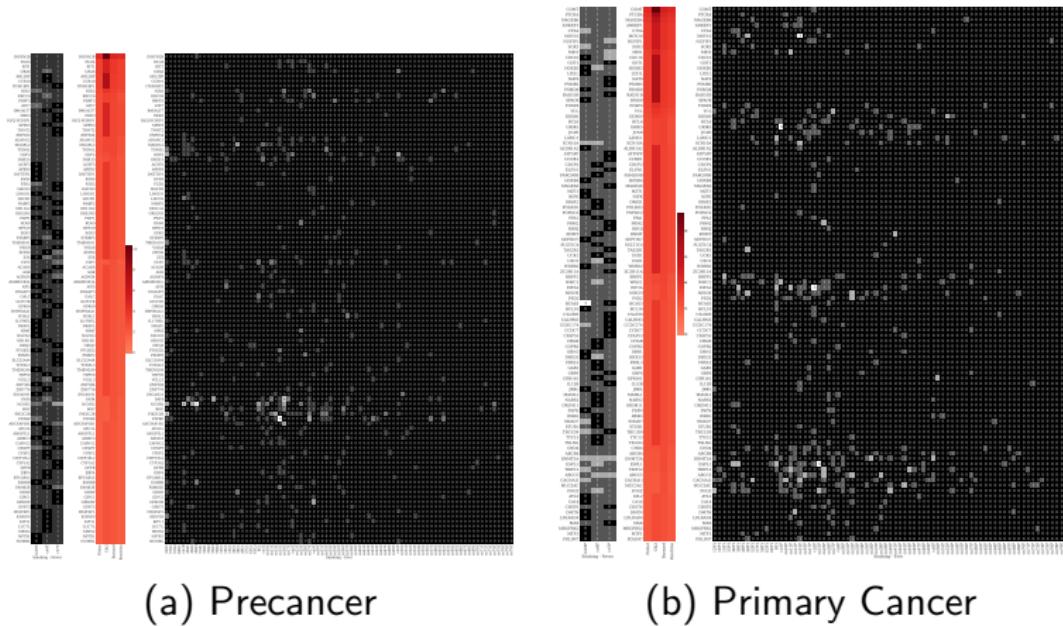


Figure: Clinical Data about LUSC for Smoking with Precancer/Primary

# Notable genes in LUSC for Smoking I

INSYN2B

content...

SGK3

content...

SCN10A

content...

INSYN2B

content...

DLG4

content...

# Notable genes in LUSC for Smoking II

RIT1

content...

COMT

content...

PTCRA

content...

MAGEB6

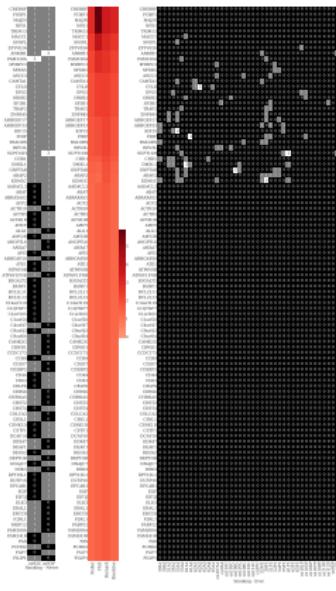
content...

# LUAD with Smoking

Table: LUAD WES Data with Smoking

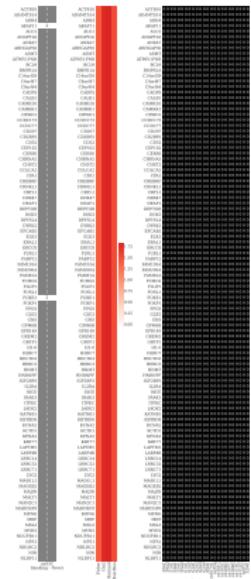
Smoking?	Stage	Number of Samples
Never	Normal	1
	CIS+AIS	1
	Primary	1
	Total	3
Ex	Normal	10
	AAH	9
	CIS+AIS	6
	Primary	10
	Total	35
Current	Normal	7
	AAH	6
	CIS+AIS	2
	MIA	1
	Primary	7
	Total	23

# Clinical Data about LUAD for Smoking I

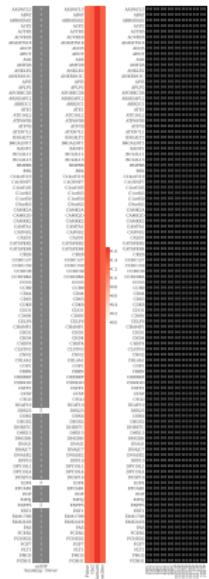


## Figure: Clinical Data about LUAD for Smoking

# Clinical Data about LUAD for Smoking II



(a) Precancer



(b) Primary Cancer

Figure: Clinical Data about LUAD for Smoking with Precancer/Primary

# Notable genes in LUAD for Smoking I

CREBRF

content...

FOXP1

content...

MAJIN

content...

ACTR10

content...

ADAMTS14

content...

# Notable genes in LUAD for Smoking II

ADH4

content...

AADACL3

content...

ABAT

content...

ABRAXAS2

content...

## 4. Results

### 4.11. Clinical Data with Point Mutation

#### 4.11.2. For Recurrence

# LUSC with Recurrence

Table: LUSC WES Data with Recurrence

Recurrence?	Stage	Number of Samples	
		Normal	Dysplasia
Recurrence	Normal	14	
	Dysplasia		4
	CIS+AIS	12	
	Primary	14	
	Total	44	
Non-recurrence	Normal	63	
	Dysplasia		1
	AAH	8	
	CIS+AIS	61	
	Primary	63	
	Total	196	

# Clinical Data about LUSC for Recurrence I

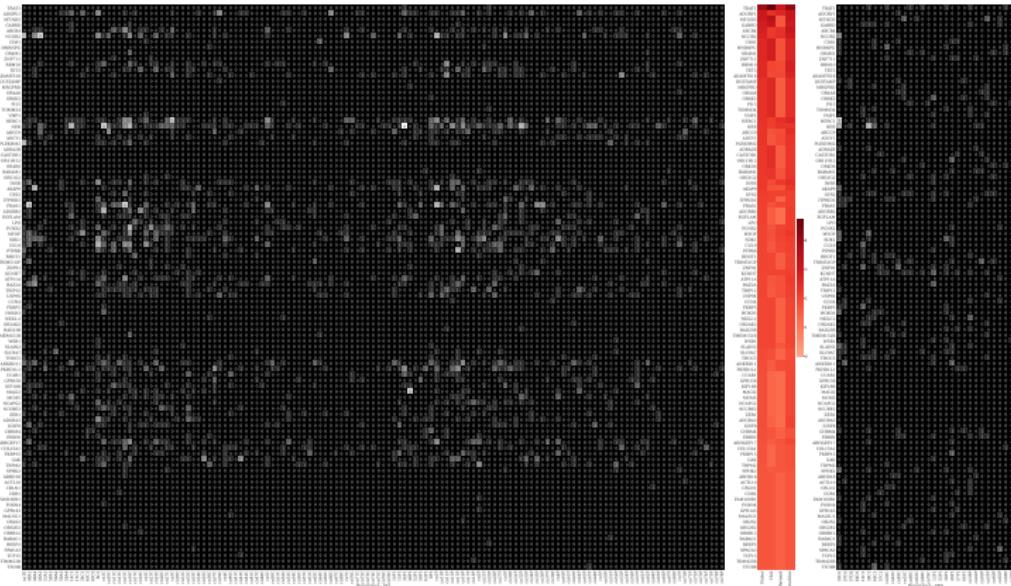


Figure: Clinical Data about LUSC for Recurrence

# Clinical Data about LUSC for Recurrence II

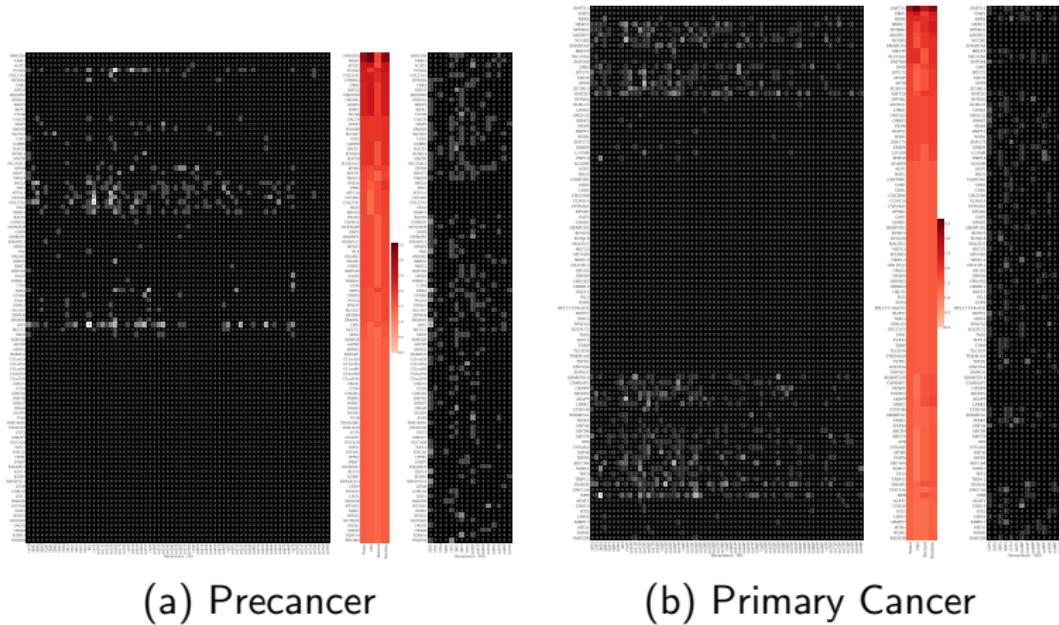


Figure: Clinical Data about LUSC for Recurrence with Precancer/Primary

# Notable genes in LUSC with Recurrence I

TRAT1

content...

ADGRV1

content...

MT-ND5

content...

HMG20A

content...

TRAT1

content...

# Notable genes in LUSC with Recurrence II

ACAT2

content...

ZNF711

content...

VMP1

content...

RERE

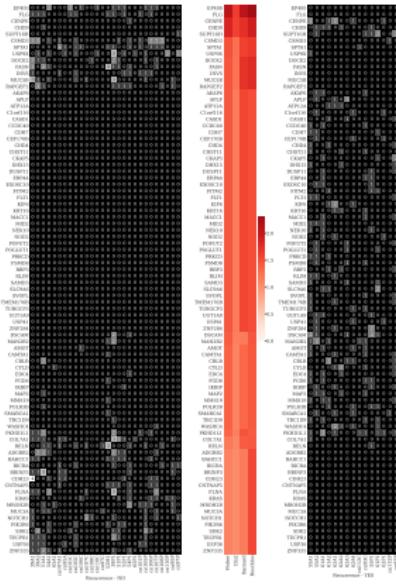
content...

# LUAD with Recurrence

Table: LUAD WES Data with Recurrence

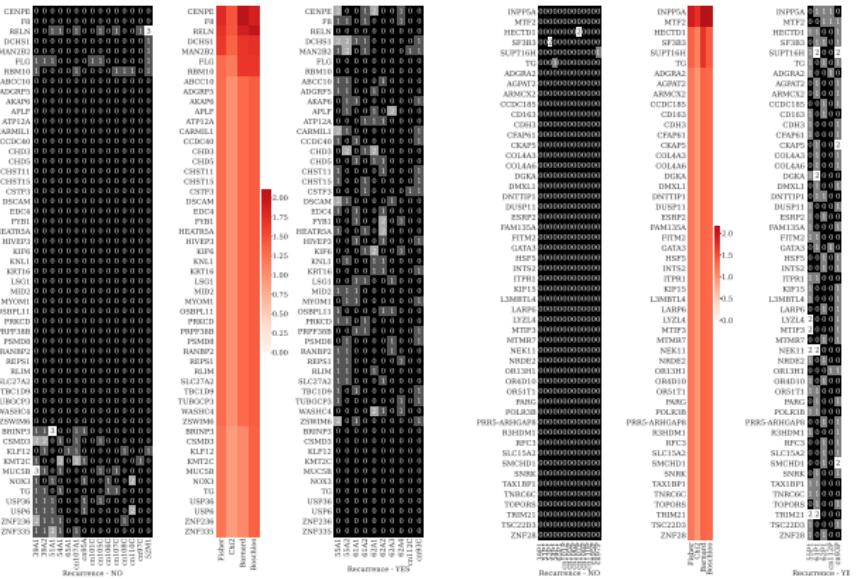
Recurrence?	Stage	Number of Samples	
		Normal	AAH
Recurrence	Normal	5	8
	AAH	2	5
	CIS+AIS	5	20
	Primary	20	
	Total		
Non-recurrence	Normal	13	7
	AAH	7	1
	CIS+AIS	13	41
	MIA		
	Primary		
	Total		

## Clinical Data about LUAD for Recurrence I



## Figure: Clinical Data about LUAD for Recurrence

## Clinical Data about LUAD for Recurrence II



(a) Precancer

(b) Primary Cancer

Figure: Clinical Data about LUAD for Recurrence with Precancer/Primary

# Notable genes in LUSC with Recurrence I

EP400

content...

FLG

content...

CENPE

content...

CENPE

content...

F8

content...

# Notable genes in LUSC with Recurrence II

RELN

content...

INPP5A

content...

MTF2

content...

HECTD1

content...

# Findings in Clinical Data with Point Mutation

## 5. Discussion

## 6. References

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