

# Lung Precancer Study

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# Overview

1 Introduction

2 Materials

3 Methods

4 Results

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# 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

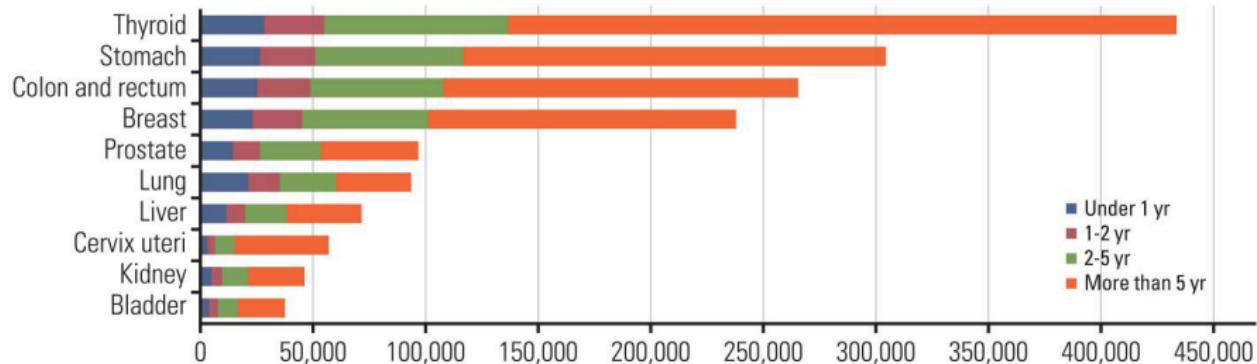


Figure: Common cancer survival rates (S. Hong et al., 2021)

## 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. LUAD

# LUAD

# 1. Introduction

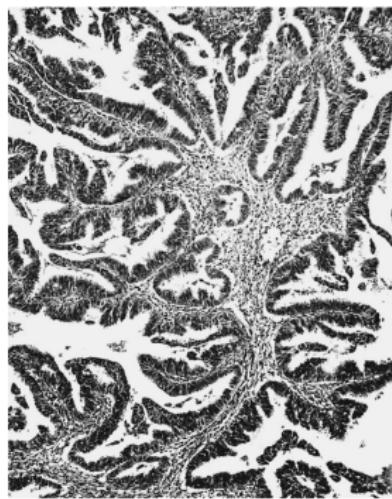
## 1.3. LUSC



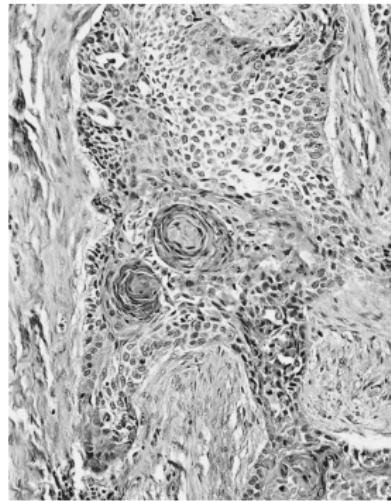
# 1. Introduction

## 1.4. LUAD vs. LUSC

# LUAD vs. LUSC I



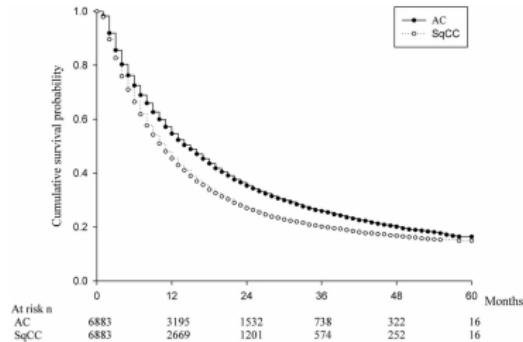
(a) LUAD



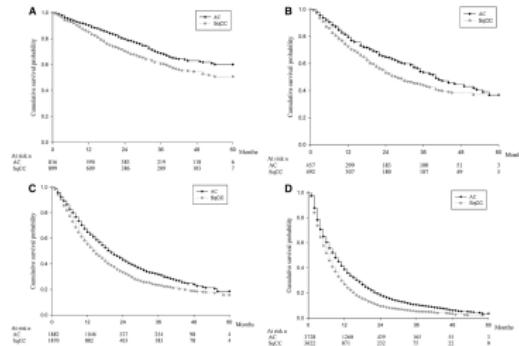
(b) LUSC

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

# LUAD vs. LUSC II



(a) All patients



(b) By cancer stages

Figure: Kaplan-Meier survival curves for LUAD & LUSC (Wang et al., 2020)

## Findings

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

# 1. Introduction

## 1.5. 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 (n=289) + Transcriptome (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?	Number of Samples	
	Stage	
Recurrence	Normal	5
	AAH	8
	CIS+AIS	2
	Primary	5
	Total	20
Non-recurrence	Normal	13
	AAH	7
	CIS+AIS	7
	MIA	1
	Primary	13
	Total	41

# WES Data Composition with Smoking I

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	

# 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	Total
LUSC	Normal	17	17
	Dysplasia	2	2
	CIS+AIS	34	34
	Primary	36	36
	Total	89	89
LUAD	Normal	13	13
	AAH	1	1
	CIS+AIS	5	5
	Primary	6	6
	Total	25	25

# WTS Data Composition with Recurrence I

Table: LUSC WTS Data with Recurrence

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 with Recurrence II

Table: LUAD WTS Data with Recurrence

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

# WTS Data Composition with Smoking I

Table: LUSC WTS Data with Smoking

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

# WTS Data Composition with Smoking II

Table: LUAD WTS Data with Smoking

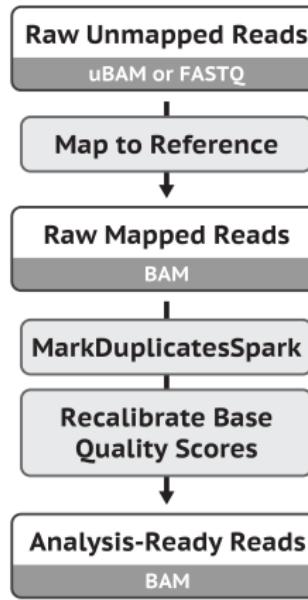
Smoking?	Stage	Number of Samples	
Never	Normal	10	
	AAH	1	
	CIS+AIS	3	
	Primary	4	
	Total	18	
Ex	Normal	3	
	CIS+AIS	1	
	Primary	1	
	Total	5	
Current	CIS+AIS	1	
	Primary	1	
	Total	2	

### 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

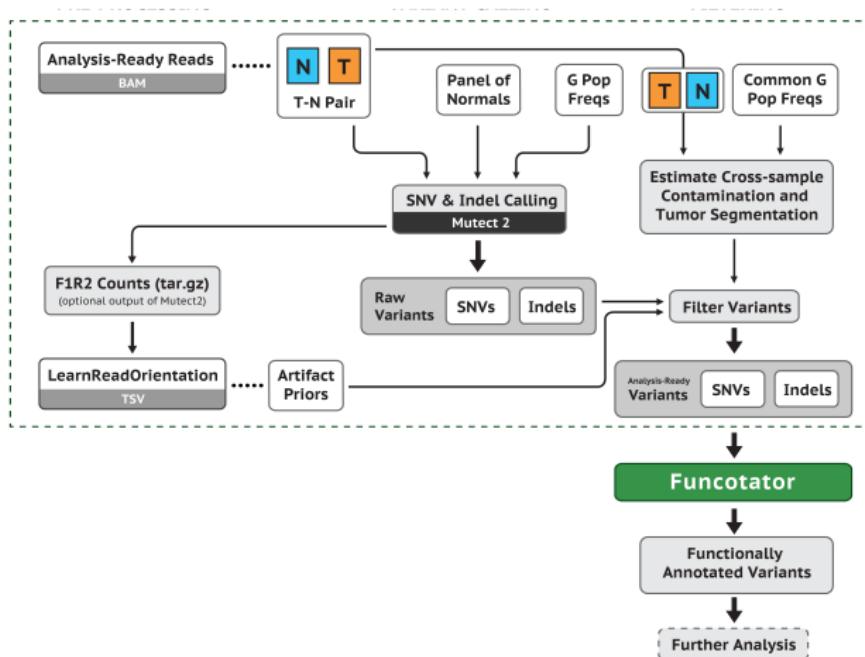
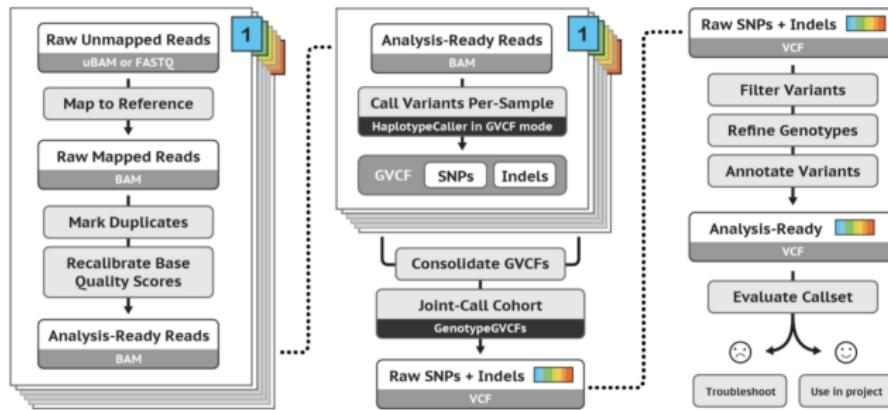


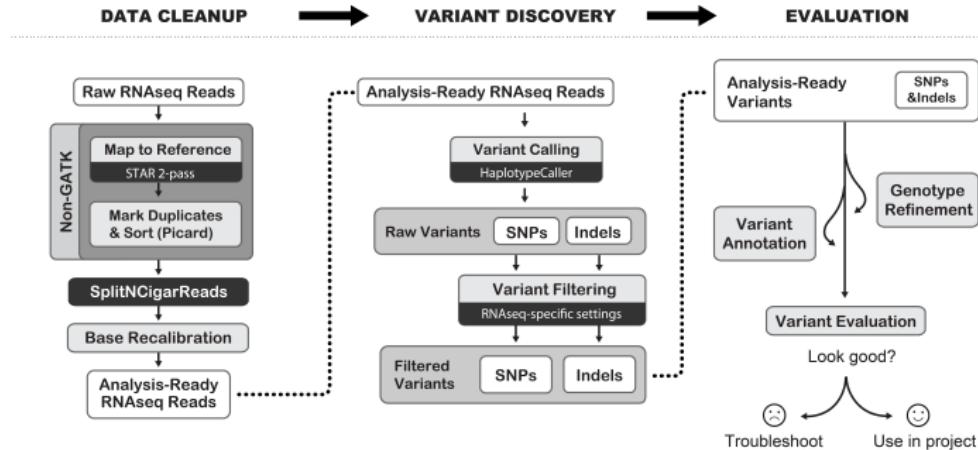
Figure: Somatic short variant (SNVs + Indels) discovery workflow (Van der Auwera et al., 2013; DePristo et al., 2011)

# 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?

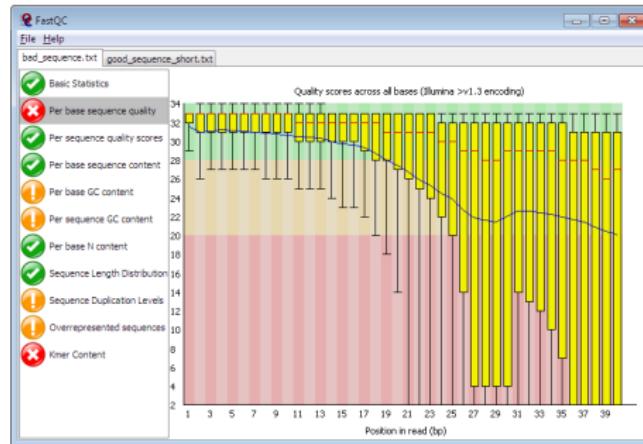


Figure: Example of FastQC Result (Andrews et al., 2012)

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

# FastQC on WES

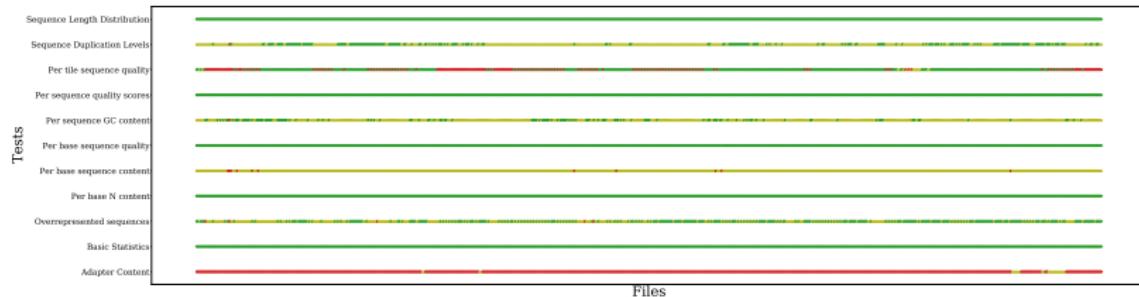
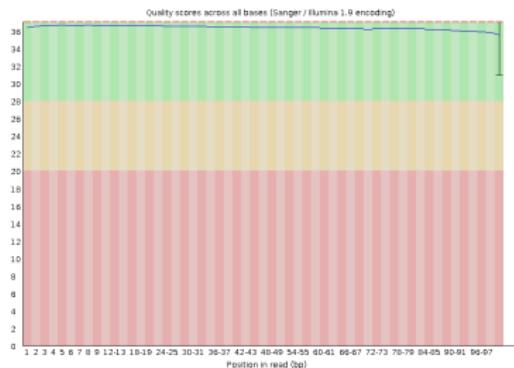


Figure: FastQC with WES data

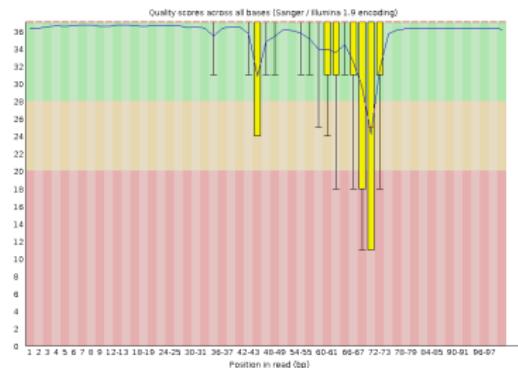
Failure on 33P1 sample

33P1 is excluded at further analysis.

# Failure on 33P1 I



(a) 33N



(b) 33P1

Figure: Per Base Sequence Quality Results

# Failure on 33P1 II

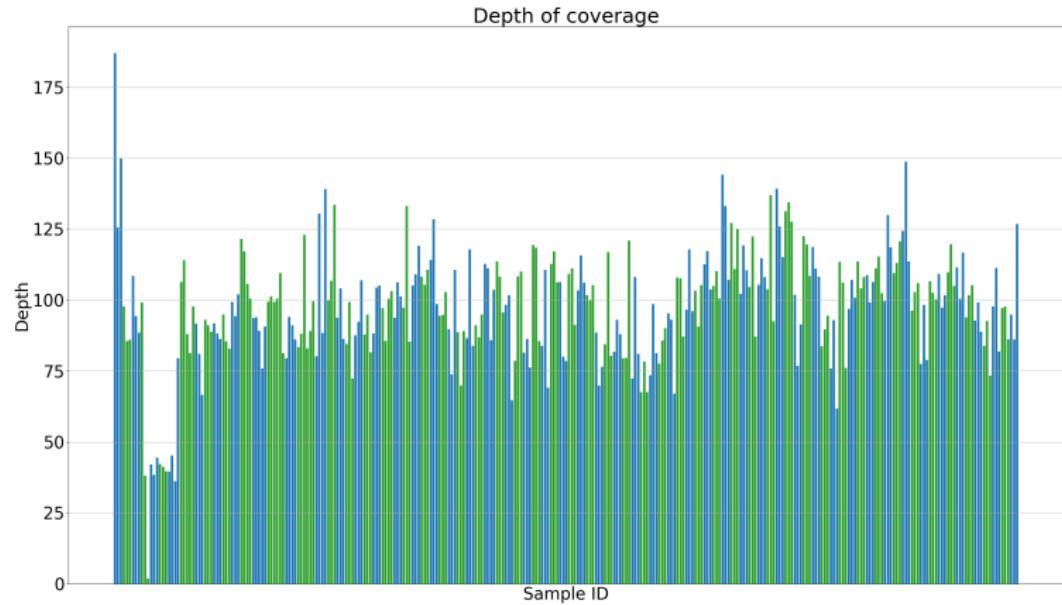


Figure: Coverage Depth Plot

## FastQC on WTS

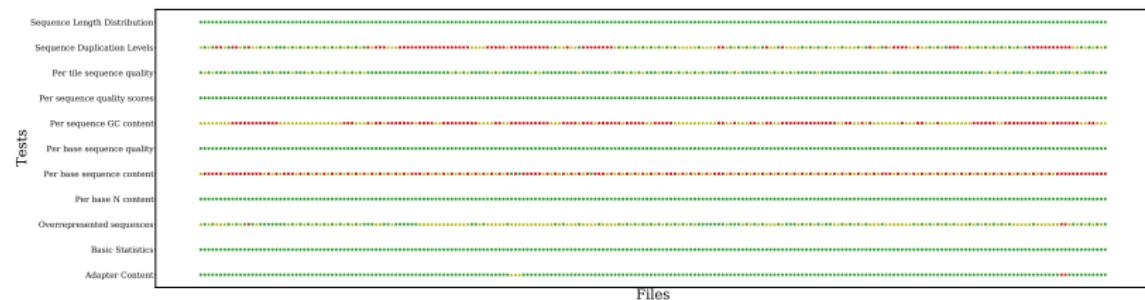


Figure: FastQC with WTS data

All sample are good to analysis

∴ No sample has more than 5 failures.

## 4. Results

### 4.2. Quality Checks with Picard

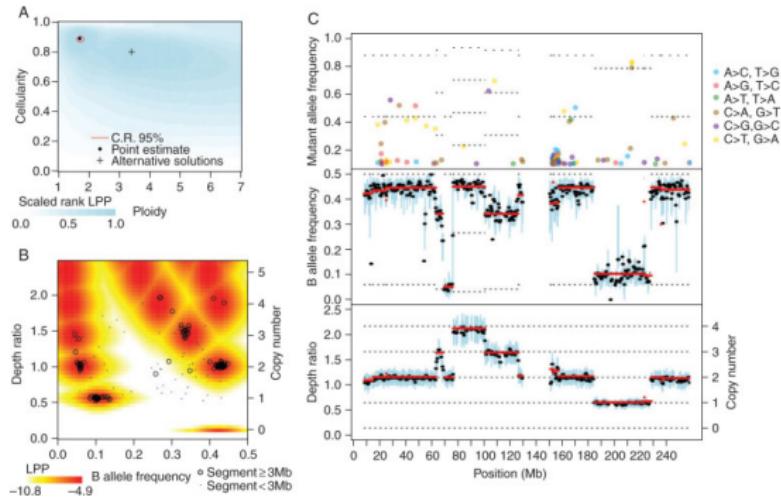
# Picard?

# Findings in Picard

## 4. Results

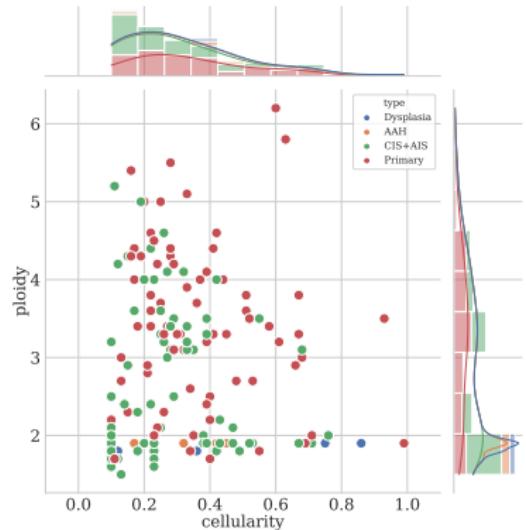
### 4.3. Copy Number Variations

# Sequenza?

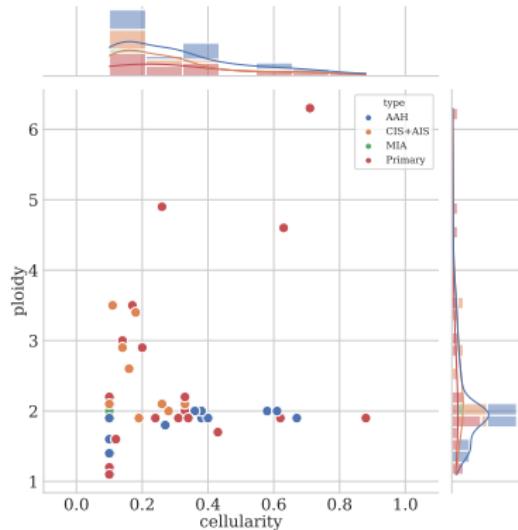


**Figure:** Representative Output of the Sequenza (Favero et al., 2015)

# Cellularity & Ploidy on WES



(a) LUSC Samples



(b) LUAD Samples

Figure: Cellularity and Ploidy from Sequenza

# LUSC in CNV Plot

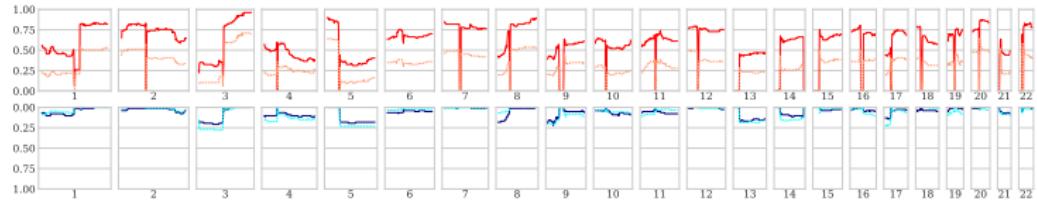


Figure: LUSC in CNV Plot

# LUSC with Recurrence in CNV Plot

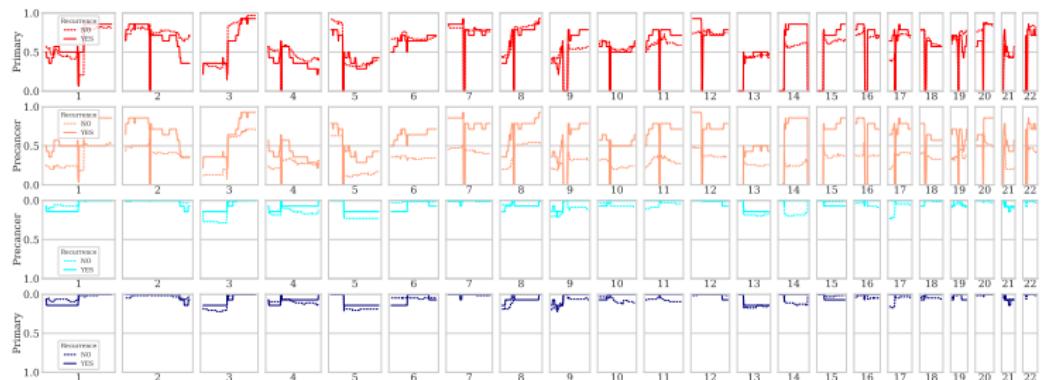


Figure: LUSC with Recurrence in CNV Plot

# LUAD in CNV Plot

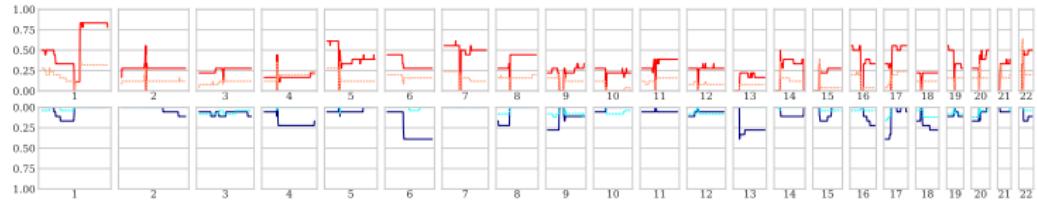


Figure: LUAD in CNV Plot

# LUAD with Recurrence in CNV Plot

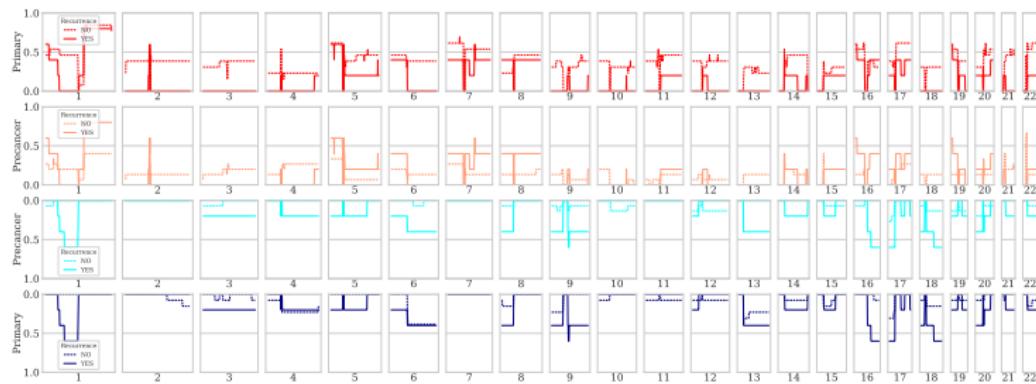


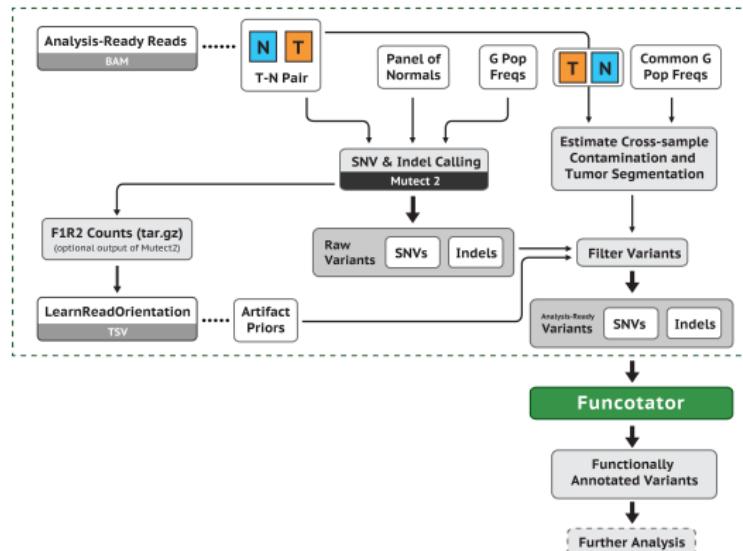
Figure: LUAD with Recurrence in CNV Plot

# Findings in Sequenza

## 4. Results

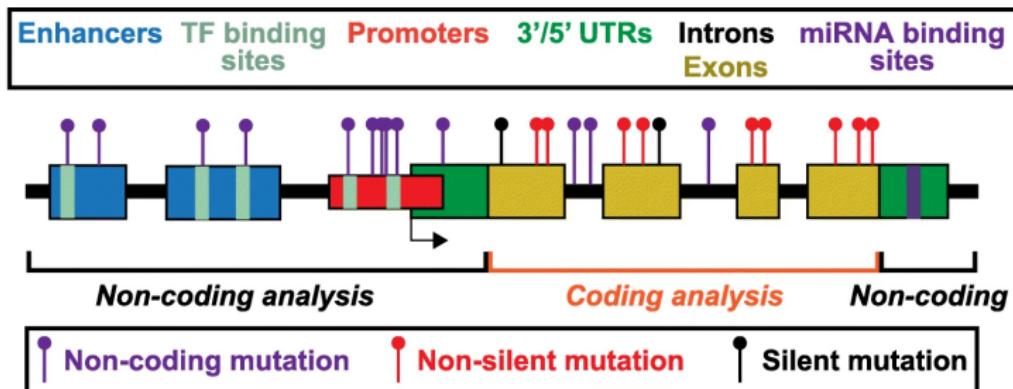
### 4.4. SNVs Analysis

# 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?

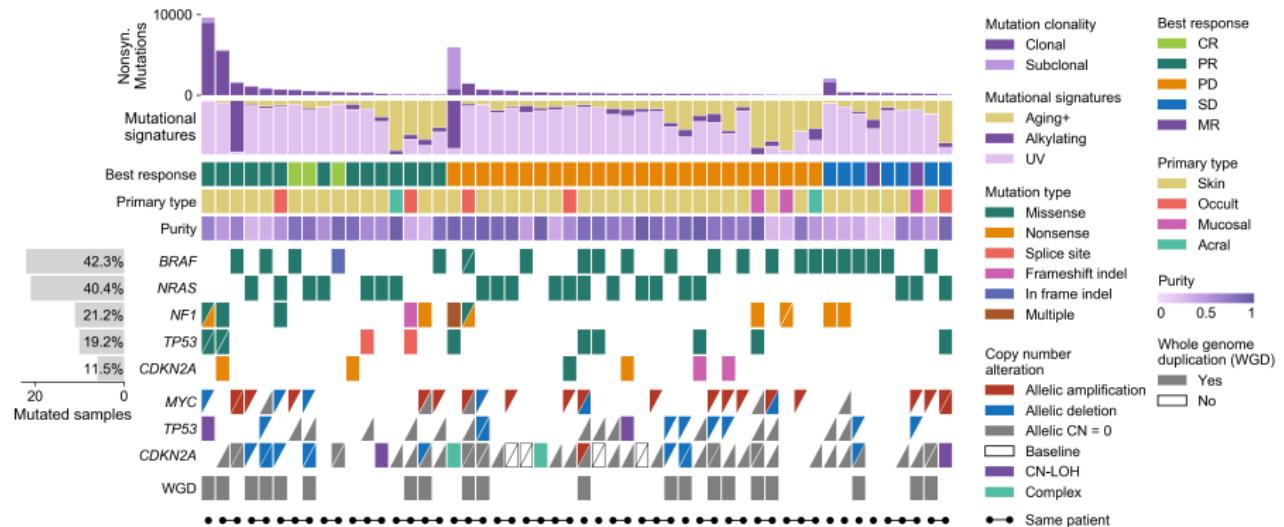


Figure: A comutation plot generated with CoMut (Crowdis et al., 2020)

# 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

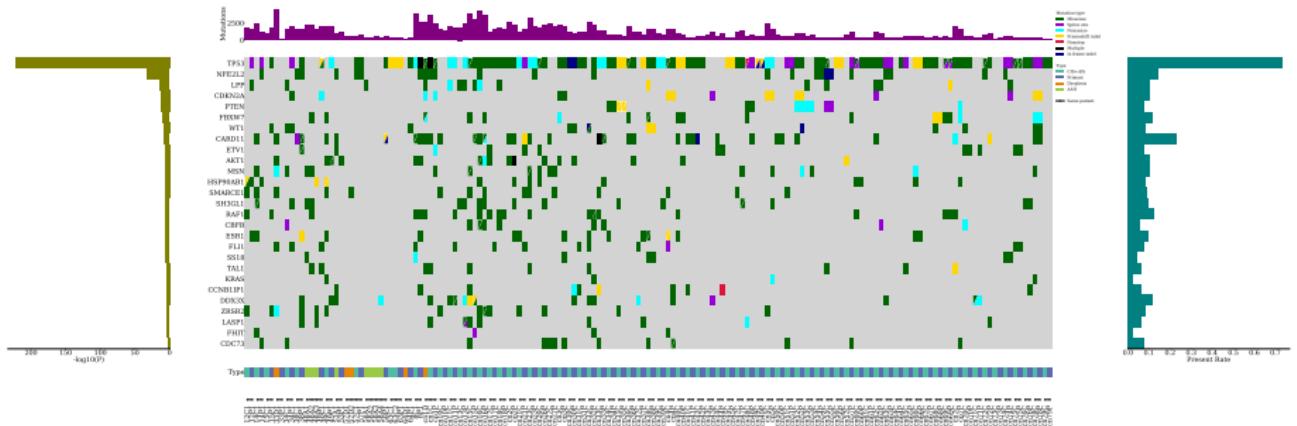


Figure: CoMut Plot with LUSC Patients

# Somatic Variant in LUSC with Recurrence

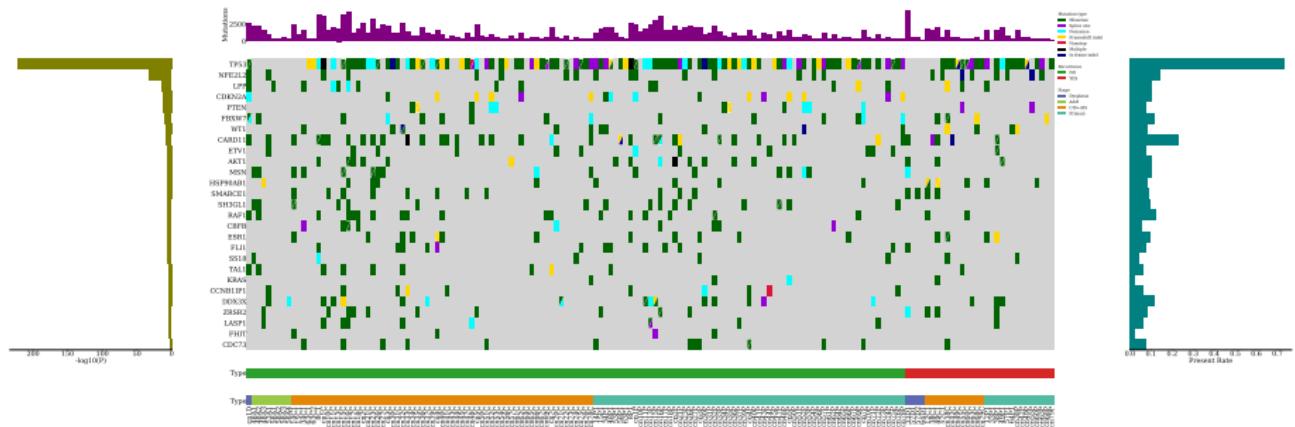


Figure: CoMut Plot in LUSC Patients with Recurrence

# Somatic Variant in LUAD

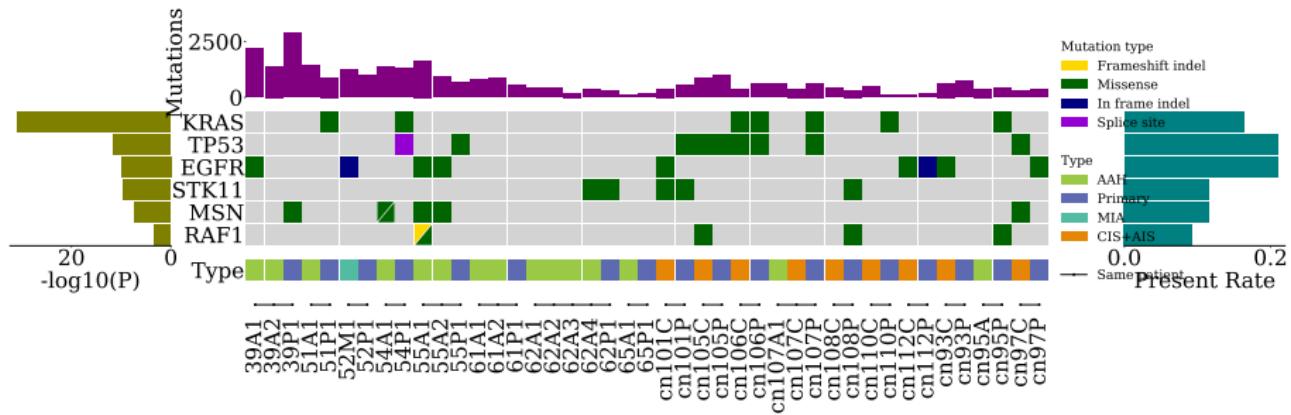


Figure: CoMut Plot with LUAD Patients

# Somatic Variant in LUAD with Recurrence

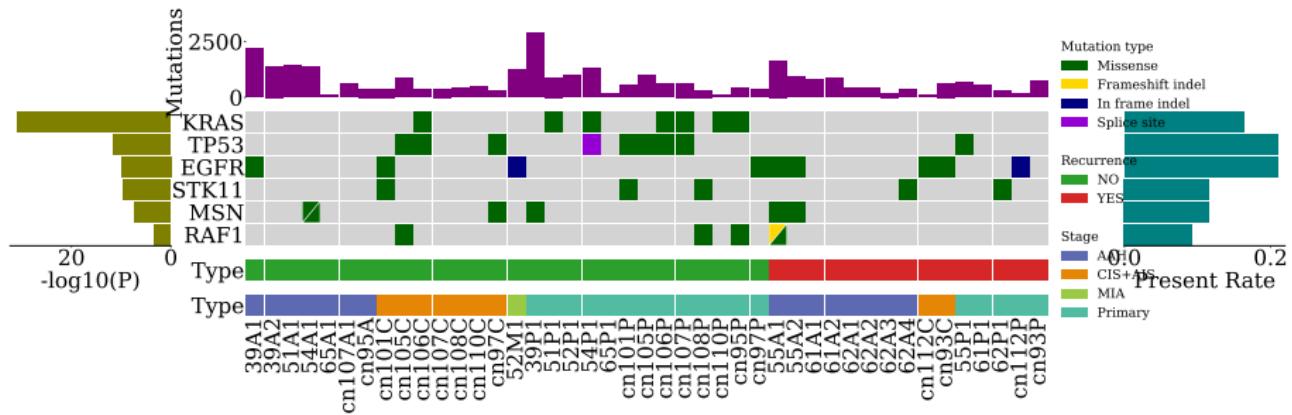


Figure: CoMut Plot in LUAD Patients with Recurrence

# 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

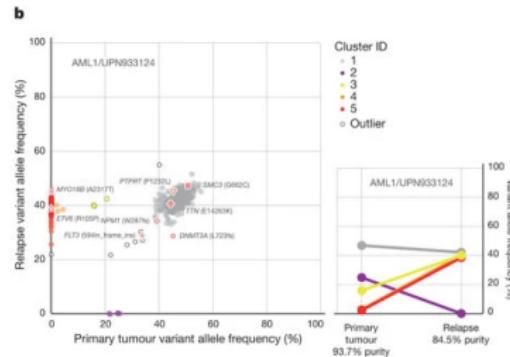


Figure: VAF distribution of validated mutations (Ding et al., 2012)

# VAF Plots I

# PyClone?

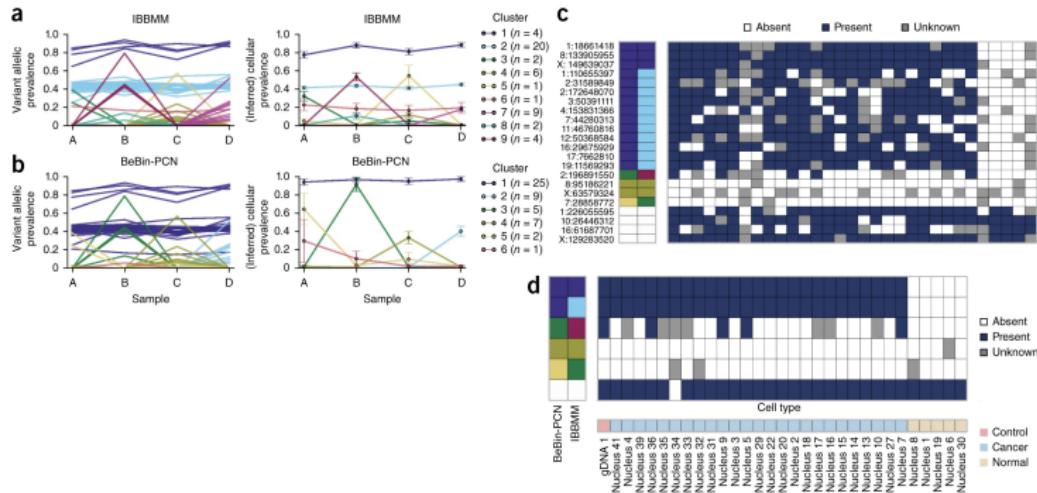


Figure: Analysis of multiple samples by PyClone (Roth et al., 2014)

# PyClone Plots I

# Findings in VAF Analysis

## 4. Results

### 4.6. Tumor Evolution Trajectories Analysis

# Revolver?

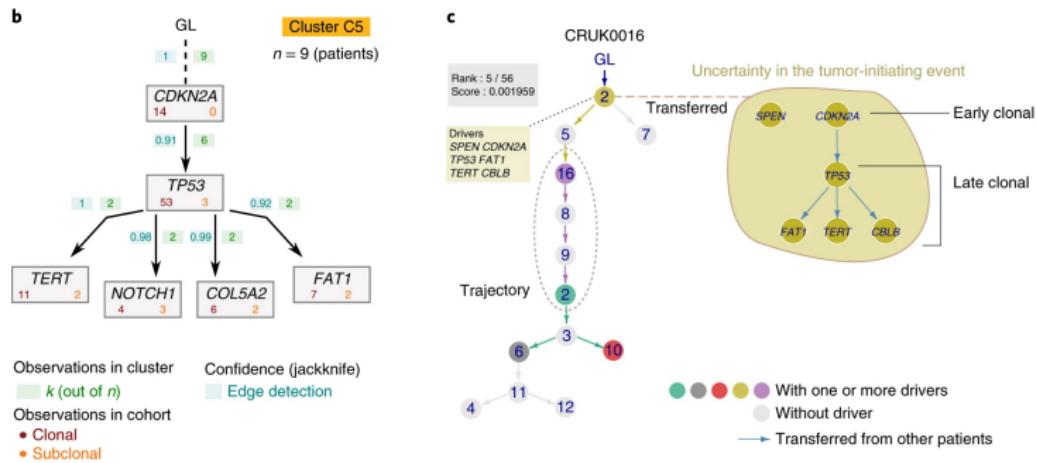


Figure: Repeated Evolutionary Trajectories (Caravagna et al., 2018)

# Findings in Tumor Evolution Trajectories Analysis

## 4. Results

### 4.7. Differences in Gene Expression Levels

# RSEM?

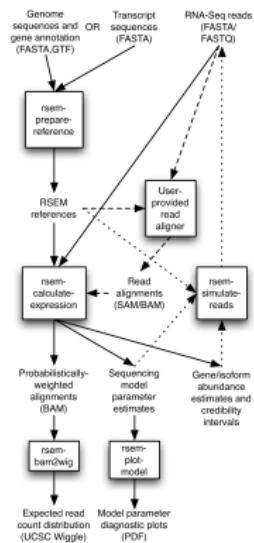


Figure: RSEM workflow (Li & Dewey, 2011)

# DESeq2?

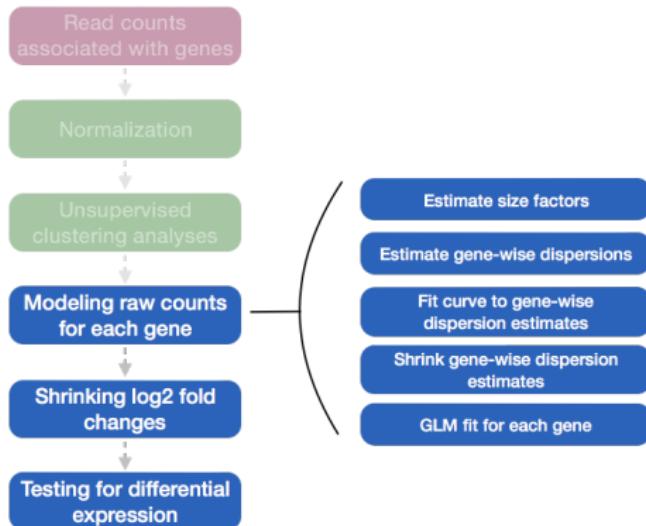


Figure: DESeq2 workflow (Love, Huber, & Anders, 2014)

# 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?

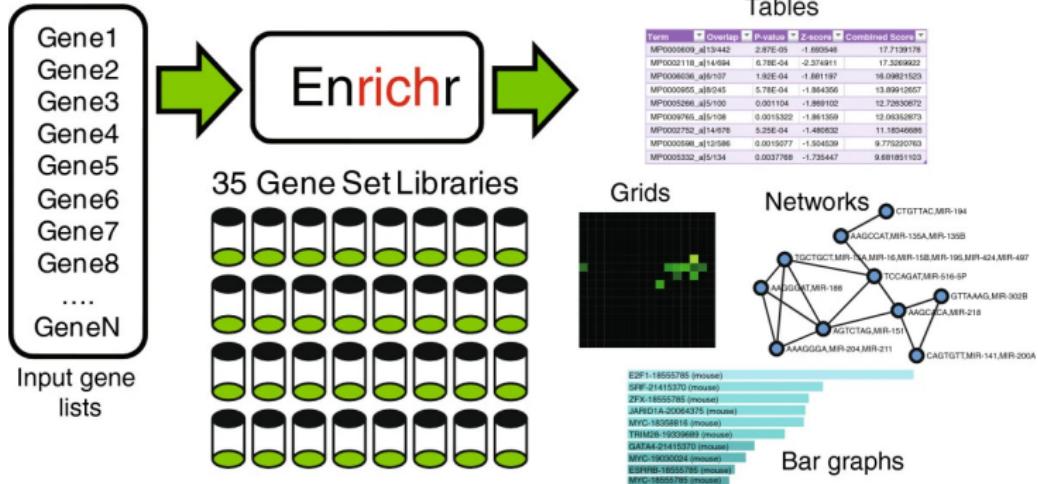


Figure: Enrichr workflow (Chen et al., 2013; Kuleshov et al., 2016)

# Gene-set Library

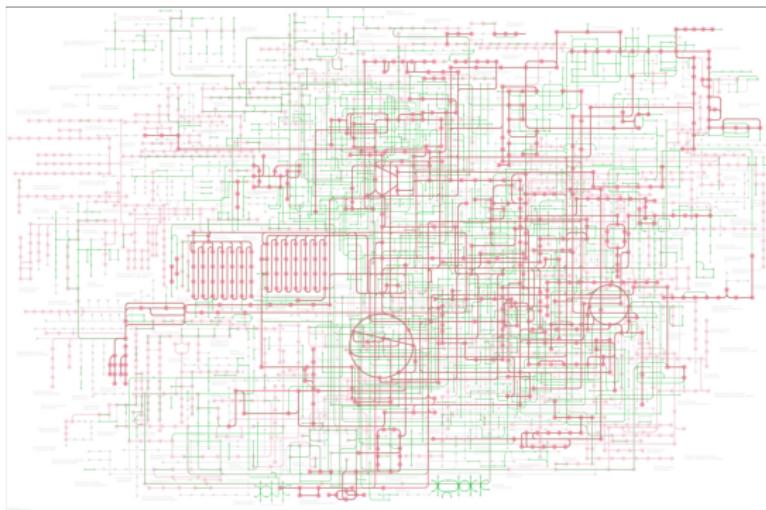


Figure: The global map of metabolic pathways by KEGG (Kanehisa et al., 2021)

KEGG

KEGG 2021 Human

# WTS Data Composition

Table: Number of WTS samples

Cancer Subtype	Stage	Number of Samples	
		Normal	17
LUSC	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	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 by Smoking I

Table: Number of WTS LUSC samples

Smoking?	Number of Samples	
	Stage	
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

## 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

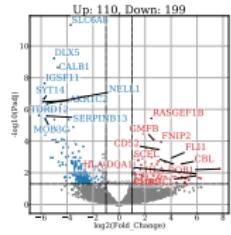
gene	log2FoldChange	pvalue	padj
AKR1C1	6.18e+00	5.14e-26	5.01e-23
AKR1C2	6.06e+00	1.19e-22	5.04e-20
CYP4F11	5.58e+00	1.51e-20	4.36e-18

Table: Down-regulated DEG in LUSC

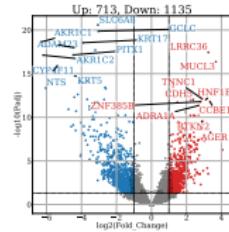
gene	log2FoldChange	pvalue	padj
SFTPC	-5.85e+00	9.16e-21	2.83e-18
FAM107A	-4.62e+00	2.27e-33	9.60e-30
LRRC36	-4.53e+00	5.49e-36	3.48e-32

## DEG Volcano Plots in LUSC

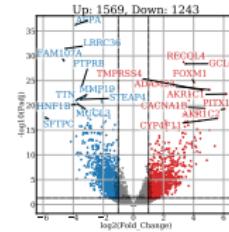
Normal → Dysplasia → CIS → Primary (LUSC)



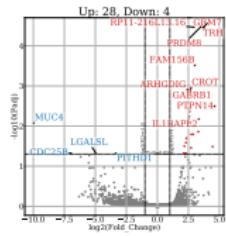
(a) Normal-Dysplasia



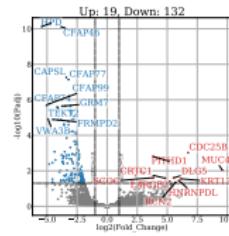
(b) Normal-CIS



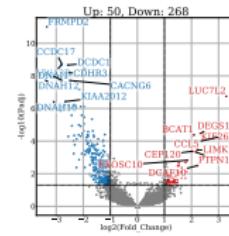
(c) Normal-Primary



(d) Dysplasia-CIS



(e) Dysplasia-Primary

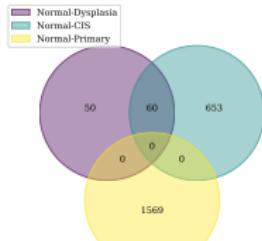


(f) CIS-Primary

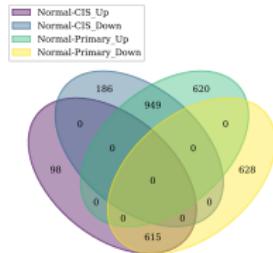
## Figure: DEG Volcano Plots in LUSC

# DEG Venn Diagram in LUSC

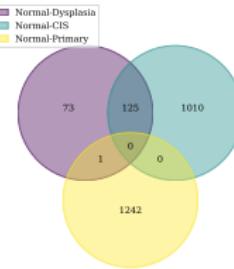
Normal → Dysplasia → CIS → Primary (LUSC)



(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	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	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	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	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	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 They are down-regulated in CIS, but up-regulated in Primary.
- ② They regulate steroids (Jin et al., 2009) and hormones (Penning et al., 2000) .
- ③ They promote the metastasis of NSCLC (Z. Hong et al., 2018).

## Down-regulation of SFTPC

- ① A pulmonary surfactant associated protein (Lin et al., 2018).
- ② SFTPC  $\downarrow \Rightarrow$  Poor survival in LUAD (Li et al., 2019).
- ③ 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

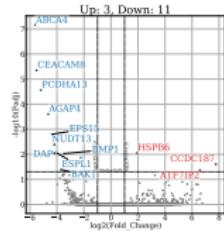
gene	log2FoldChange	pvalue	padj
ABCA4	4.95e+00	3.01e-12	2.58e-09
HMGA2	4.79e+00	8.06e-08	1.46e-05
KIF12	4.48e+00	1.33e-06	1.46e-04

Table: Down-regulated DEG in LUAD

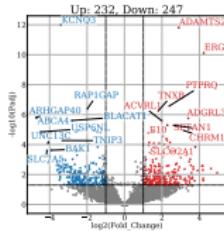
gene	log2FoldChange	pvalue	padj
SLC6A4	-6.20e+00	5.80e-10	2.36e-07
IL1RL1	-4.20e+00	7.47e-06	5.82e-04
RNF185	-4.06e+00	4.75e-05	2.45e-03

# DEG Volcano Plots in LUAD

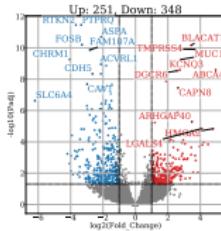
Normal → AAH → AIS → Primary (LUAD)



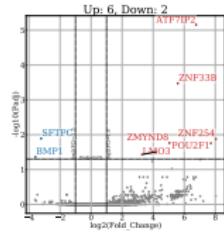
### (a) Normal-AAH



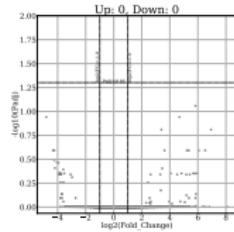
(b) Normal-AIS



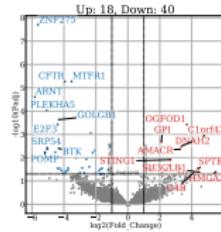
(c) Normal-Primary



(d) AAH-AIS



(e) AAH-Primary

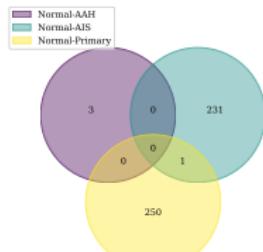


(f) AIS-Primary

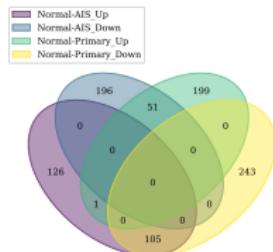
## Figure: DEG Volcano Plots in LUAD

# DEG Venn Diagram in LUAD

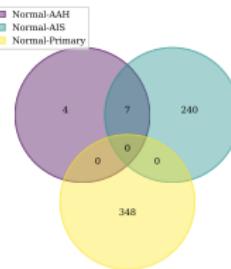
Normal → AAH → AIS → Primary (LUAD)



(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	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 disease (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

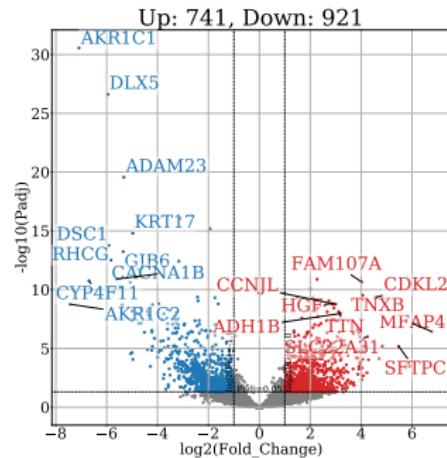
Recurrence?	Stage	Number of Samples
Recurrence	Normal	1
	Dysplasia	1
	CIS+AIS	5
	Primary	6
	Total	13
Non-recurrence	Normal	16
	Dysplasia	1
	CIS+AIS	29
	Primary	30
	Total	76

## Pooled normal samples

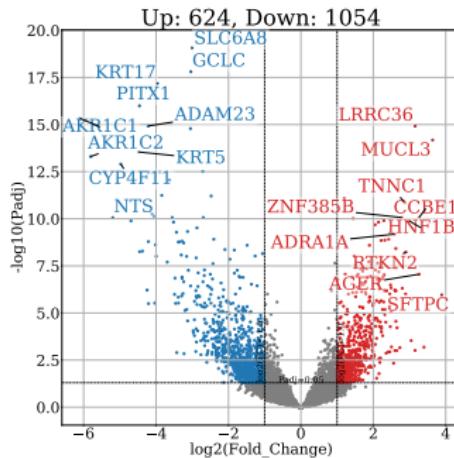
In order to compare with Normal stage, merging Normal samples.

∴ Insufficient number of Normal samples in Recur.

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



(a) Recur



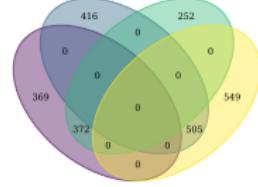
(b) Non-recur

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	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	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

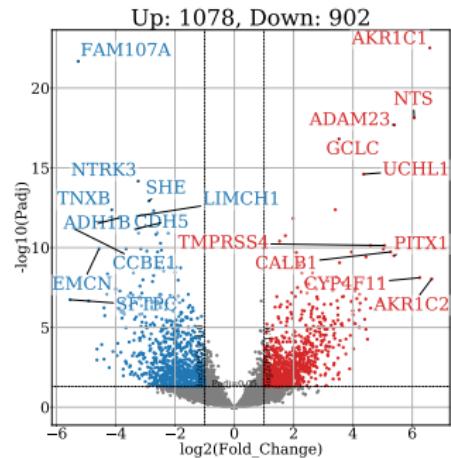
Table: Up-regulated Pathways for Intersected

Term name	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

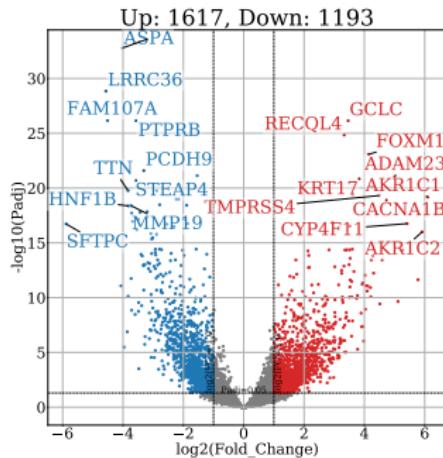
Table: Down-regulated Pathways for Intersected

Term name	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



(a) Recur



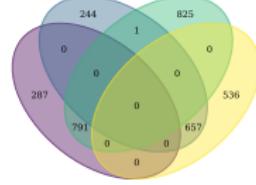
(b) Non-recur

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	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	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	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	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.

## ASPA

- ① Highly down-regulated in Non-recur patients.

## 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

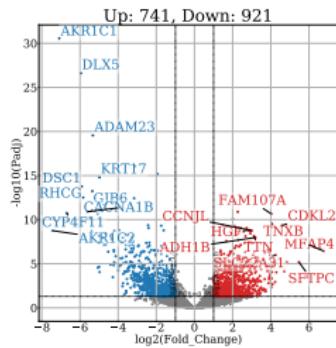
Table: Up-regulated DEG for Primary within Recur in LUSC

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

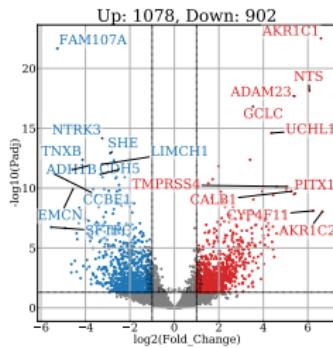
Table: Down-regulated DEG for Primary within Recur in LUSC

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

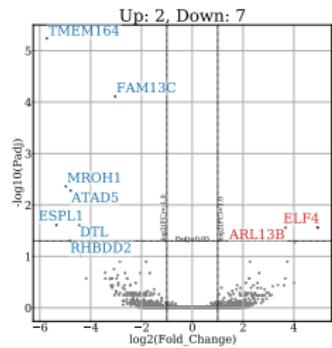
# DEG Volcano Plots with Recur in LUSC



(a) Normal-CIS



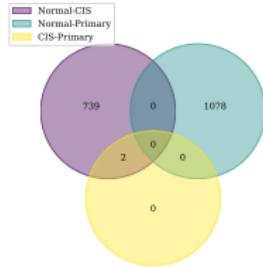
(b) Normal-Primary



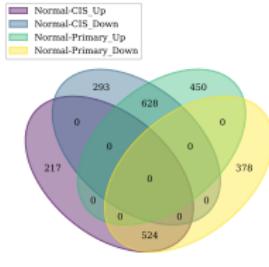
(c) CIS-Primary

Figure: DEG Volcano Plots with Recur samples in LUSC

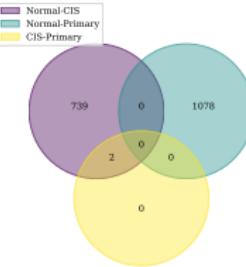
# DEG Venn Diagram with Recur in LUSC



(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	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	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	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	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.

## ADAM23

- ① Down-regulated in CIS, but up-regulated in Primary.

## FAM107A

- ① Up-regulated in CIS, but down-regulated in Primary.

## 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-recr in LUSC

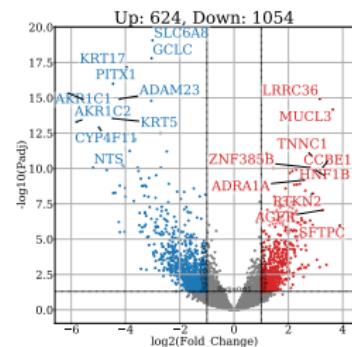
Table: Up-regulated DEG for Primary within Non-recr in LUSC

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

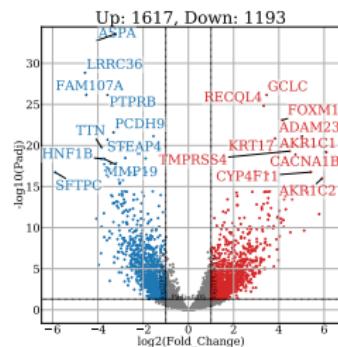
Table: Down-regulated DEG for Primary within Non-recr in LUSC

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

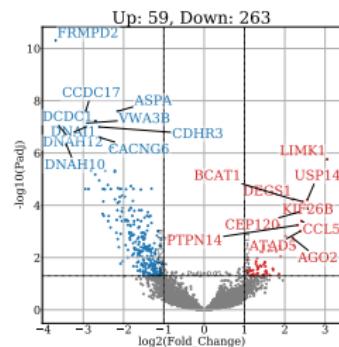
## DEG Volcano Plots with Non-recur in LUSC



(a) Normal-CIS



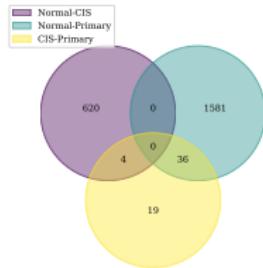
(b) Normal-Primary



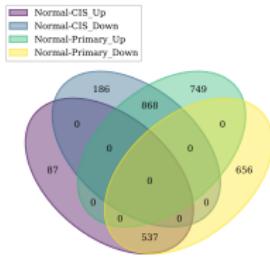
(c) CIS-Primary

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

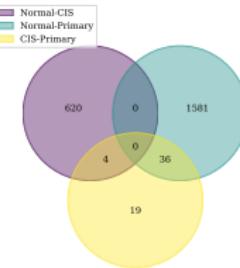
# DEG Venn Diagram with Non-recur in LUSC



(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	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	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	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	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-recr in LUSC I

## AKR1C1 & AKR1C2

- ① Down-regulated in CIS, but up-regulated in Primary.

## CYP4F11

- ① Down-regulated in CIS, but up-regulated in Primary.

## LRRC36

- ① Up-regulated in CIS, but down-regulated in Primary.

## 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	
		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	

# 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

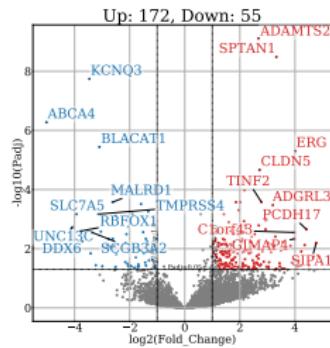
Table: Up-regulated DEG for Primary within Non-recur in LUAD

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

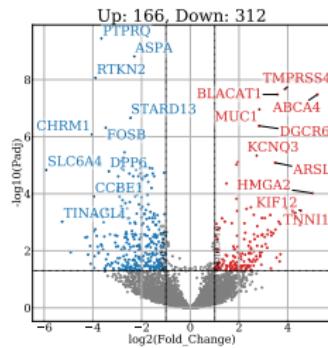
Table: Down-regulated DEG for Primary within Non-recur in LUAD

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

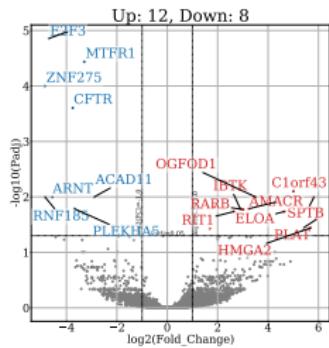
# DEG Volcano Plots with Non-recr in LUAD



(a) Normal-AIS



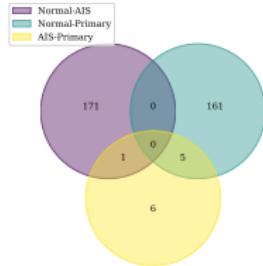
(b) Normal-Primary



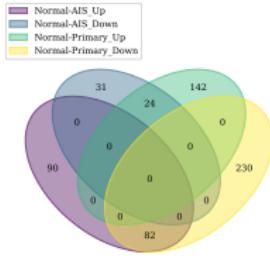
(c) AIS-Primary

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

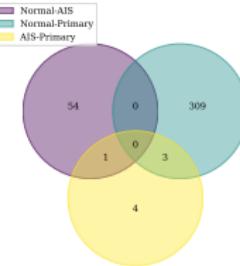
# DEG Venn Diagram with Non-recur in LUAD



(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	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

# Finding in Comparing within Non-recur in LUAD I

## KCNQ3

- ① Down-regulated in AIS, but up-regulated in Primary.

## BLACAT1

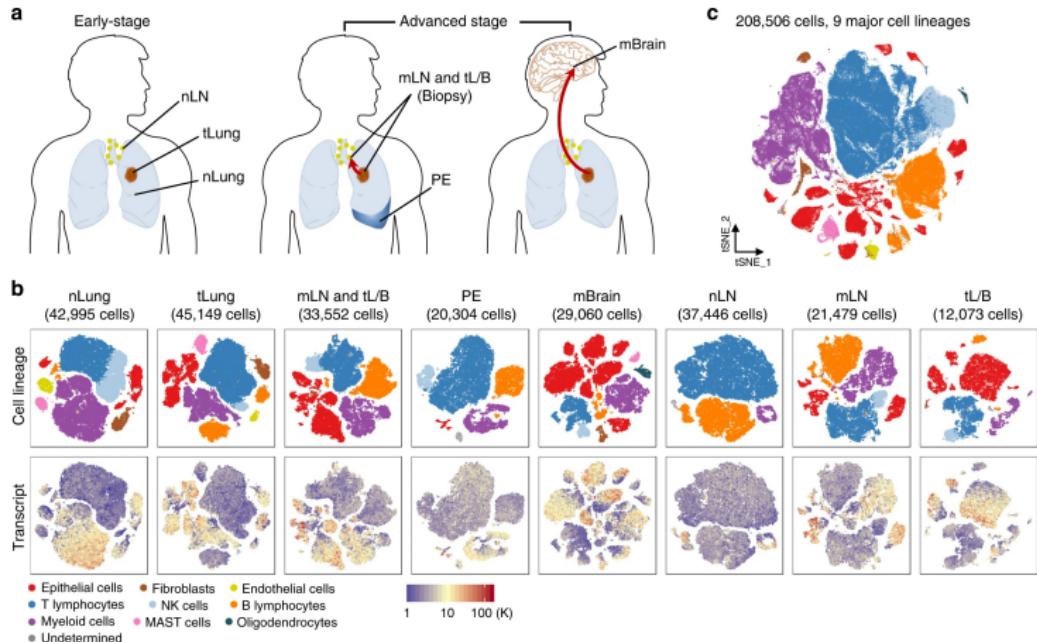
- ① Down-regulated in AIS, but up-regulated in Primary.

# Findings in DEG Analysis

## 4. Results

### 4.8. Bulk Cell Deconvolution

# Single-cell data as Reference



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

# BisqueRNA?

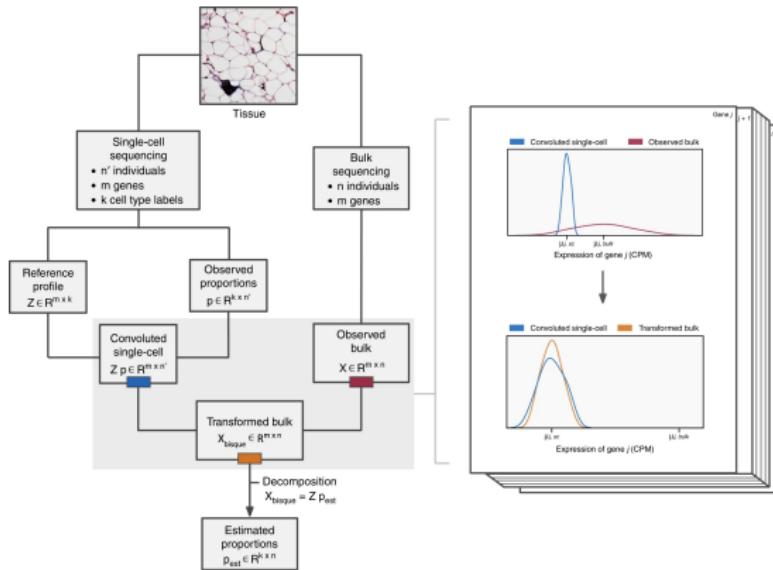


Figure: Workflow for BisqueRNA (Jew et al., 2020)

# Cluster Plot in LUSC

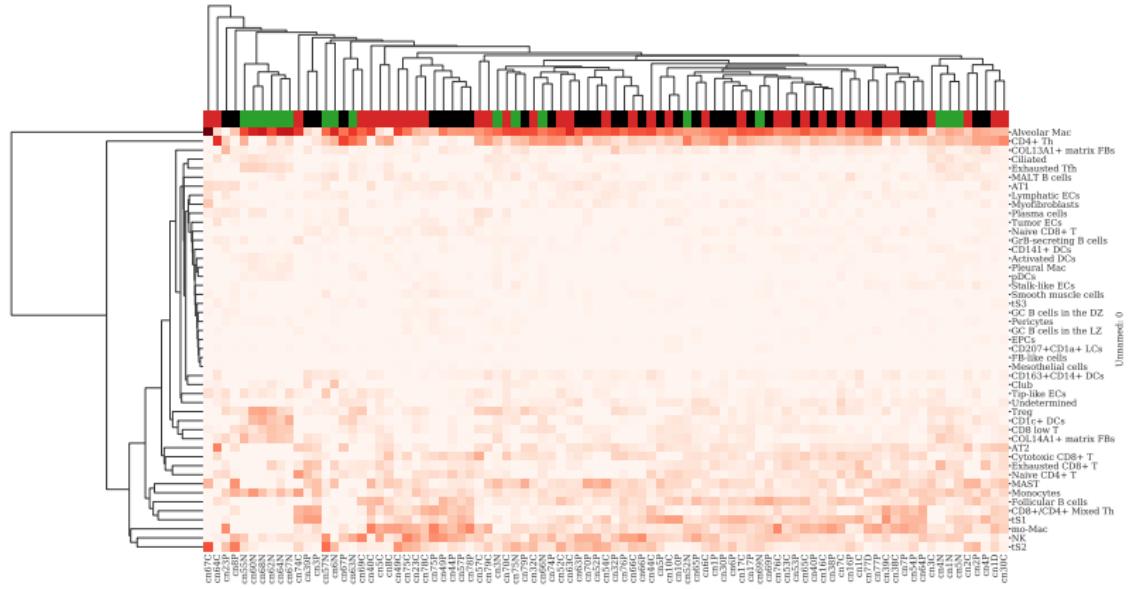
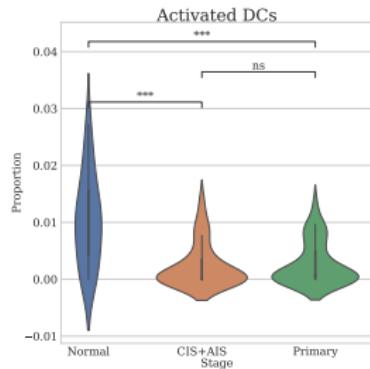
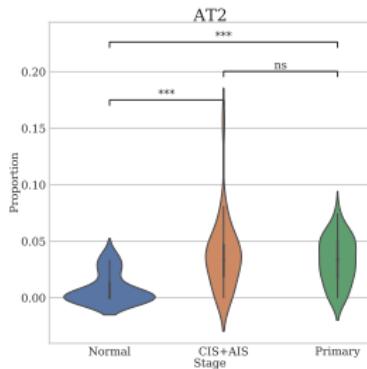


Figure: Cluster Plot in LUSC

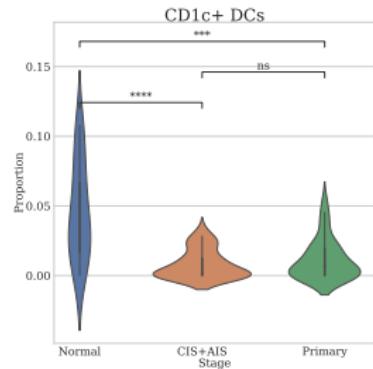
# Violin Plots in LUSC I



(a) Activated DCs



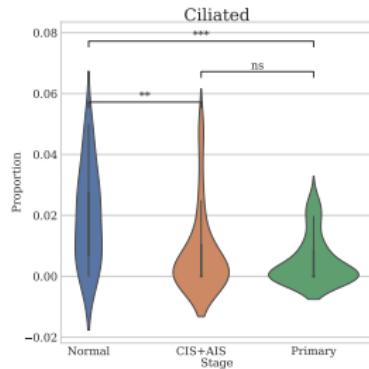
(b) Alveolar type II



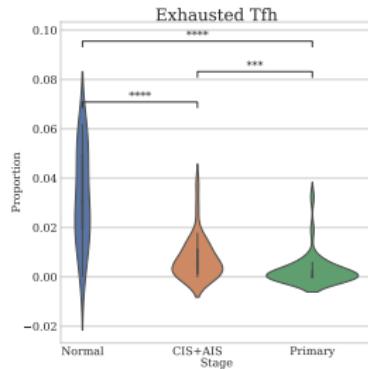
(c) Langerhans cells

Figure: Violin Plots in LUSC

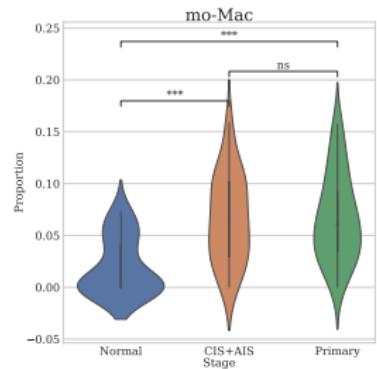
# Violin Plots in LUSC II



(d) Ciliated cells



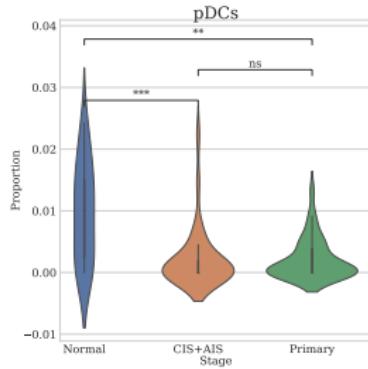
(e) Exhausted T follicular helper



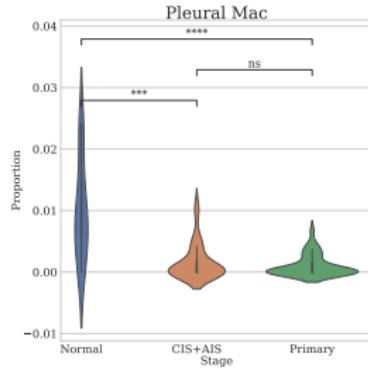
(f) Mo & Mac

Figure: Violin Plots in LUSC

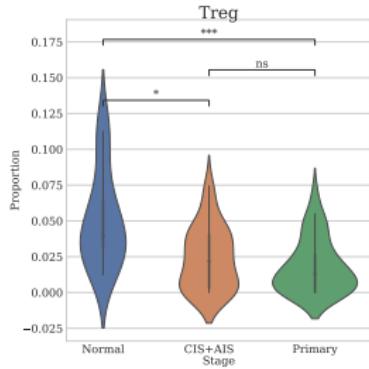
# Violin Plots in LUSC III



(g) Plasmacytoid DCs



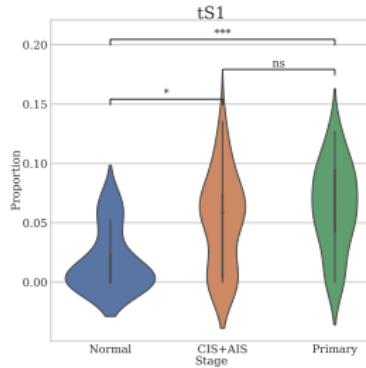
(h) Pleural Mac



(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

content...

Alveolar type II

content...

CD1c+ DCs (Langerhans cells)

content...

Ciliated cells

content...

Exhausted T follicular help

content...

# Findings in Bulk Cell Deconvolution with LUSC II

Monocyte & Macrophage  
content...

Transcriptional states 1  
content...

# Cluster Plot in LUAD

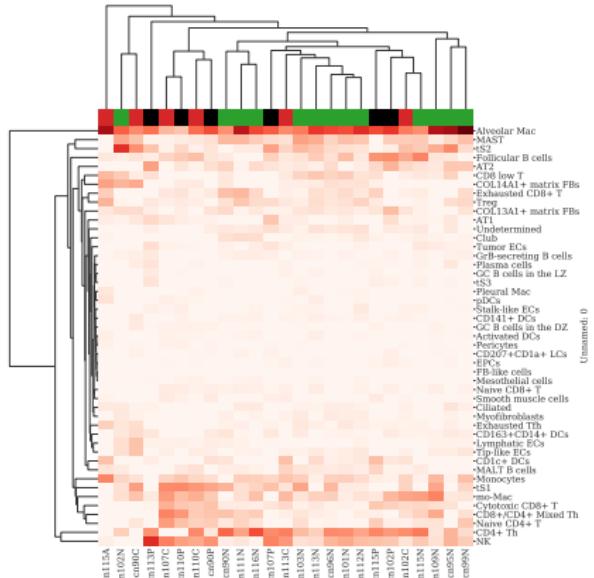
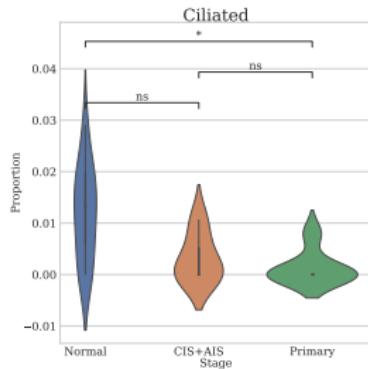
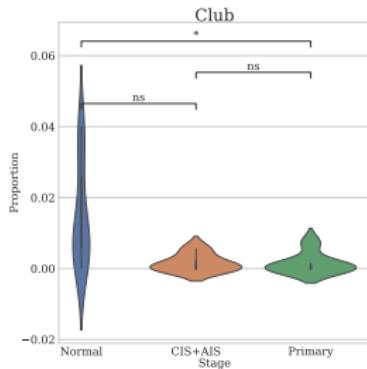


Figure: Cluster Plot in LUAD

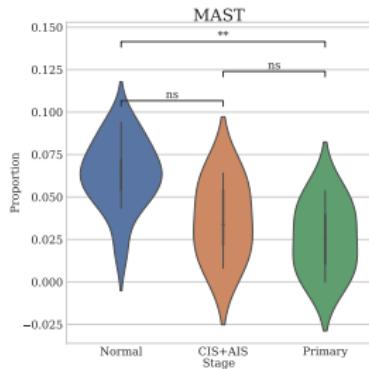
# Violin Plots in LUAD I



(a) Ciliated cells



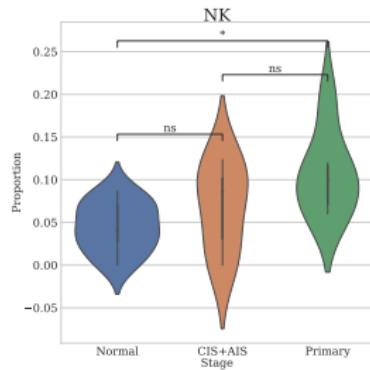
(b) Club Cell



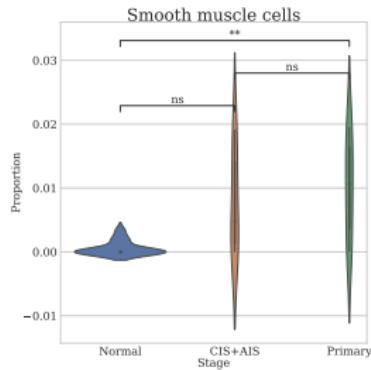
(c) Mast cell

Figure: Violin Plots in LUAD

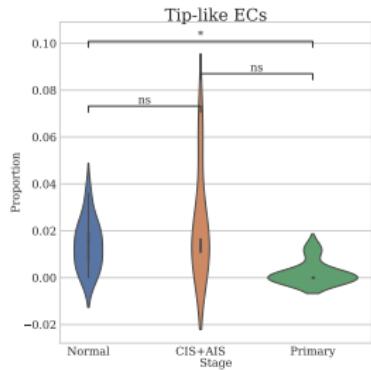
# Violin Plots in LUAD II



(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

content...

Club cells

content...

Mast cells

content...

Natural Killer cells

content...

Smooth muscle cells

content...

# Findings in Bulk Cell Deconvolution with LUAD II

Tip-like ECs

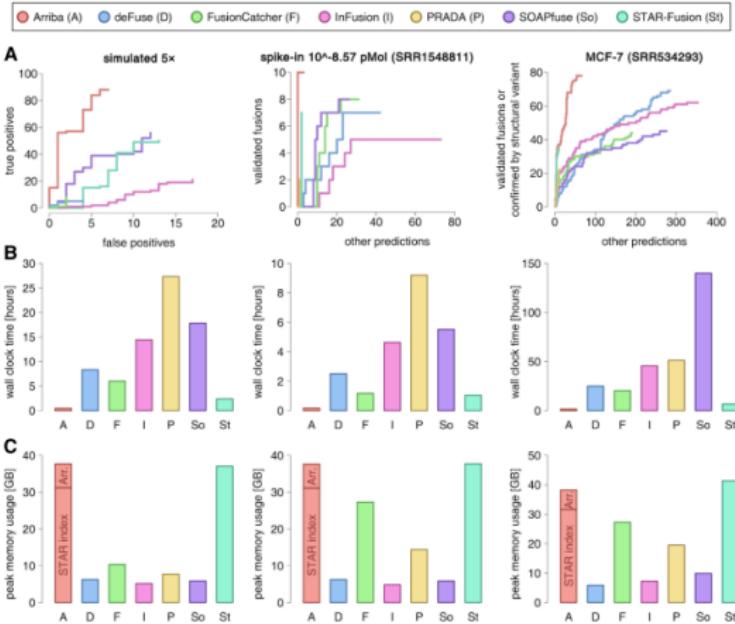
content...

# 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 Cancer Signature

# Cancer Signature?

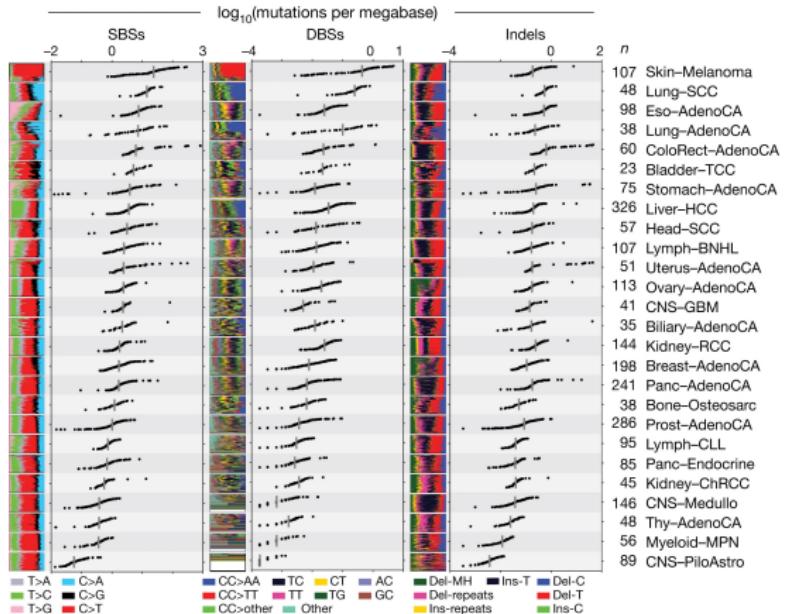
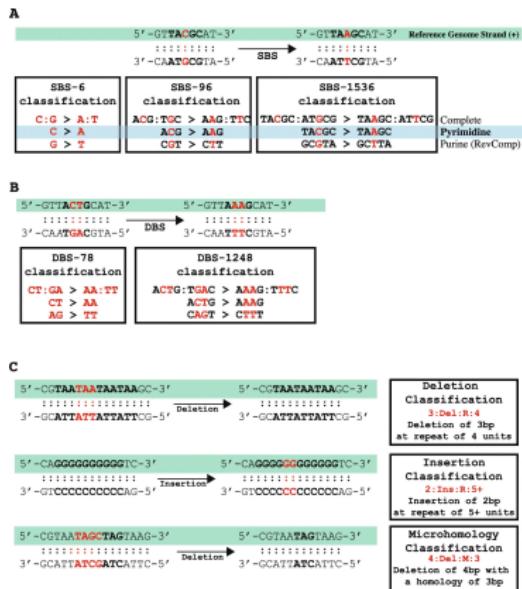


Figure: Mutation Burdens across PCAWG tumor types (Alexandrov et al., 2020)

# SigProfiler?



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

# Findings in Cancer Signature

## 4. Results

### 4.11. Clinical Data with Point Mutation

# Mutect2?

# Findings in Clinical Data with Point Mutation

## 5. Discussion

## 6. References

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