

# Lung Precancer Study

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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

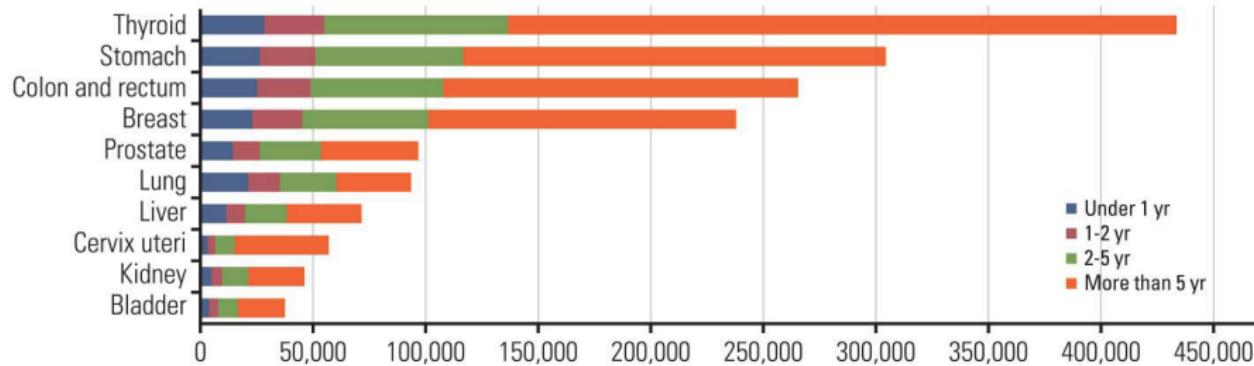


Figure: Common cancer survival rates (S. Hong et al., 2021)

## Survival rate (More than 5 yr)

- Thyroid: 68.4 %
- Lung: 35.4 %

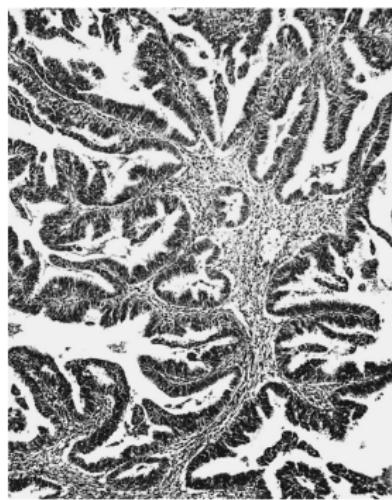
# Type of Lung Cancer

Types of lung cancer:

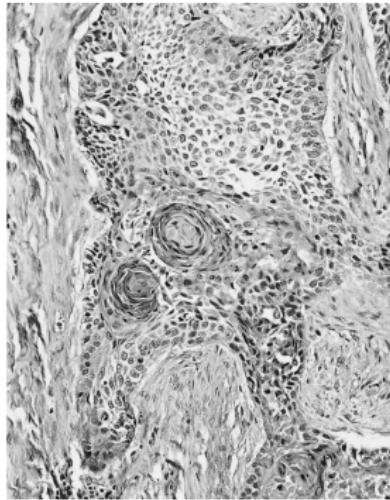
- ① Adenocarcinoma (LUAD) (40 %) ★
- ② Squamous cell carcinoma (LUSC) (25 %) ★
- ③ Small cell carcinoma (20 %)
- ④ Large cell carcinoma (10 %)
- ⑤ Adenosquamous carcinoma (< 5 %)
- ⑥ Carcinoid (< 5 %)
- ⑦ Bronchioalveolar (Bronchial gland carcinoma)

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

# LUAD vs. LUSC I



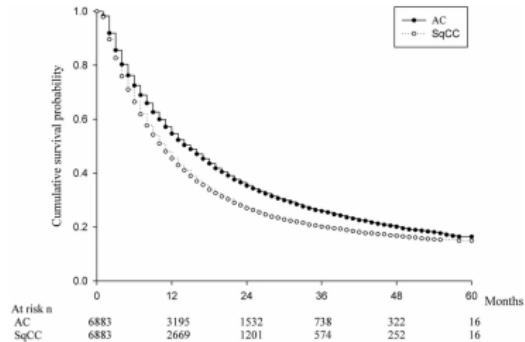
(a) LUAD



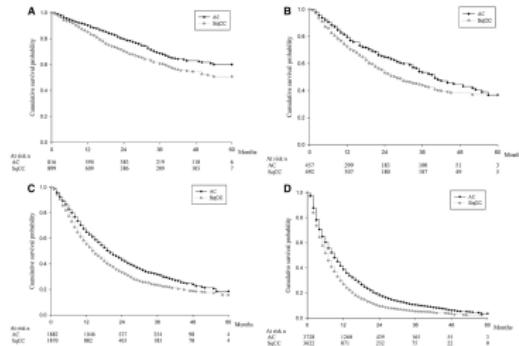
(b) LUSC

**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

### 1.2. 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

- WES (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)

### 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

# 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

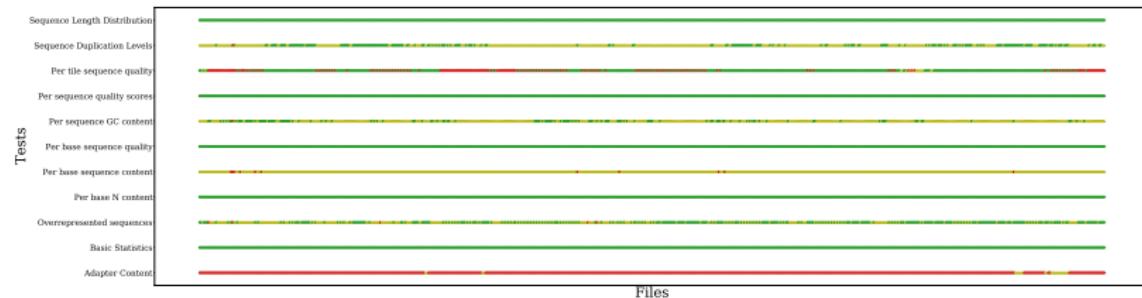
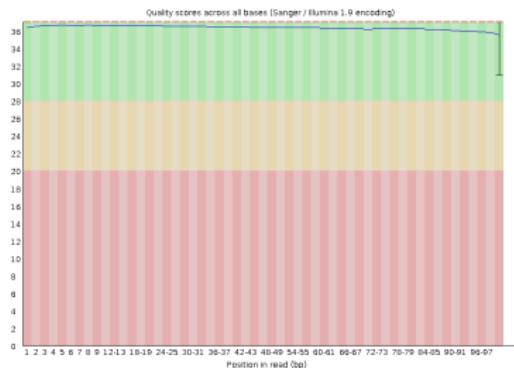


Figure: FastQC with WES data

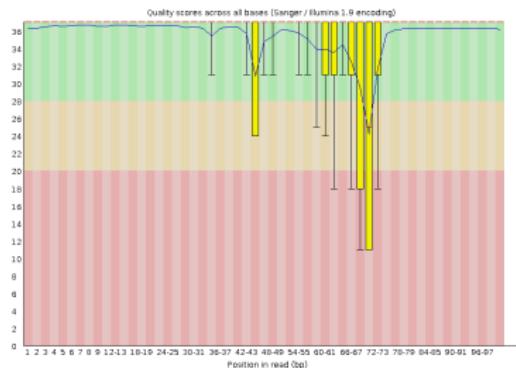
Failure on 33P1 sample

33P1 is excluded at further analysis.

# Failure on 33P1 I



(a) 33N



(b) 33P1

Figure: Per Base Sequence Quality Results

# Failure on 33P1 II

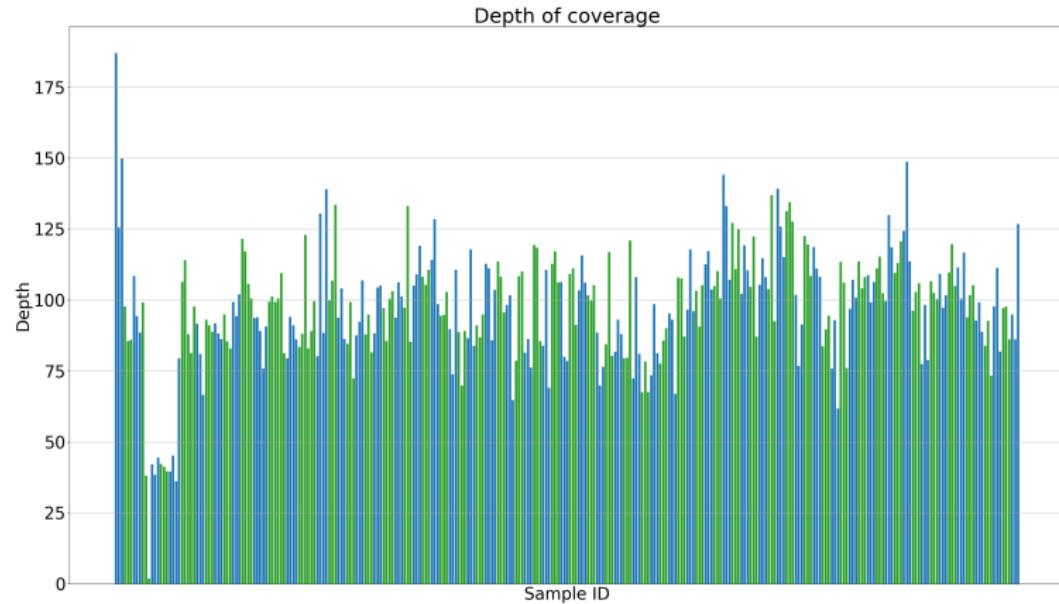


Figure: Coverage Depth Plot

# FastQC on WTS

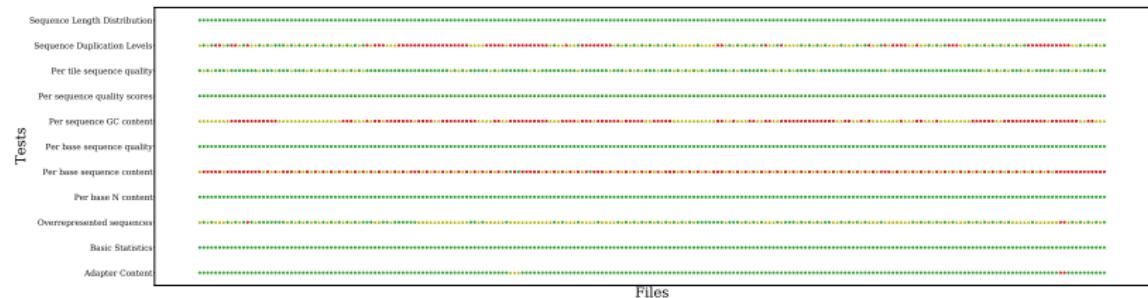


Figure: FastQC with WTS data

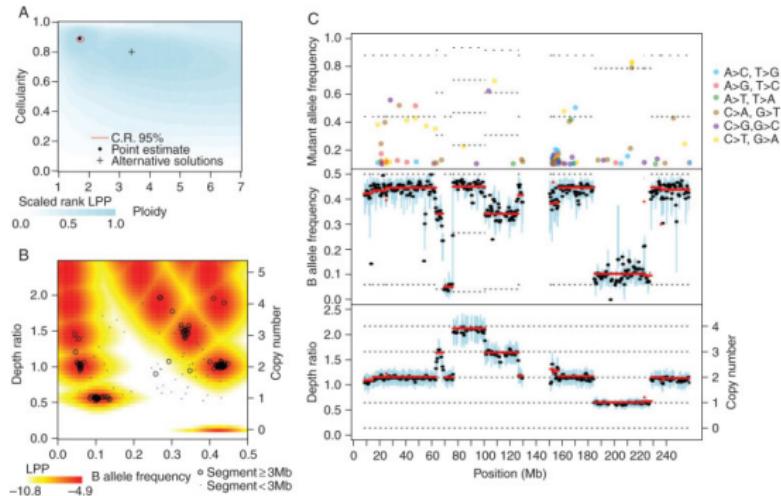
All sample are good to analysis

∴ No sample has more than 5 failures.

## 4. Results

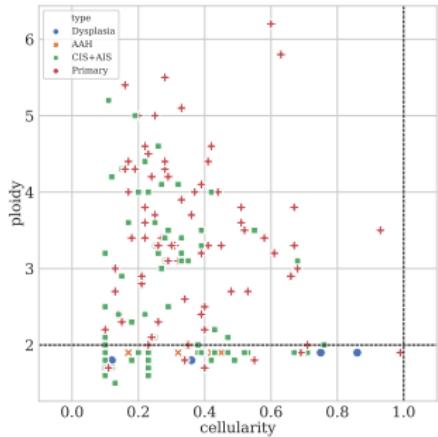
### 4.2. Copy Number Variations

# Sequenza?

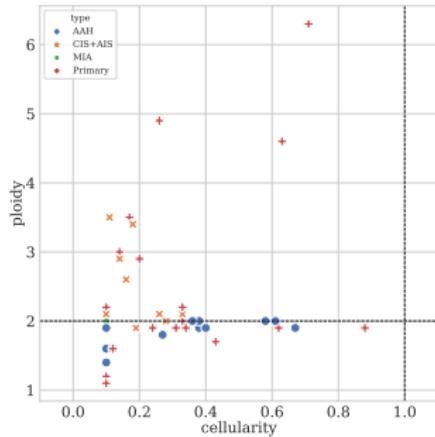


**Figure:** Representative Output of the Sequenza (Favero et al., 2015)

# Cellularity & Ploidy on WES



(a) LUSC Samples



(b) LUAD Samples

Figure: Cellularity and Ploidy from Sequenza

# Genome View on Patient #57

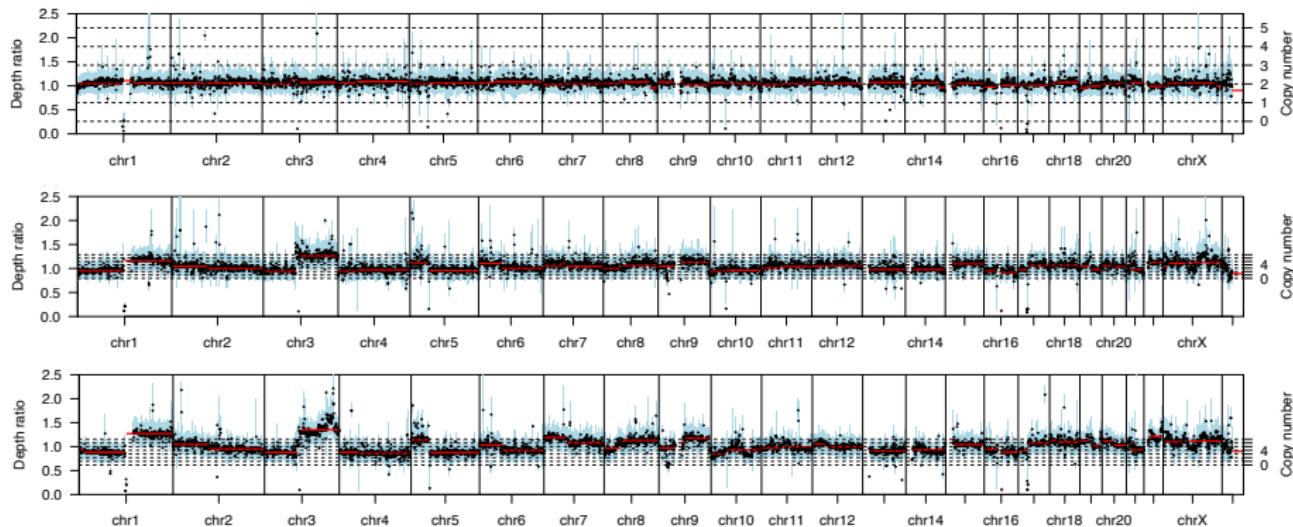


Figure: Dysplasia-CIS-Primary Tumor on Patient #57

# CNVs of LUSC

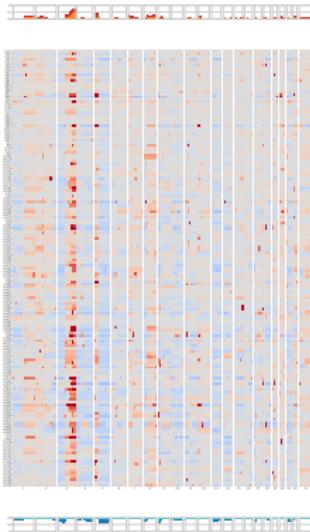


Figure: CNV Plot with LUSC Patients

# CNVs of LUAD

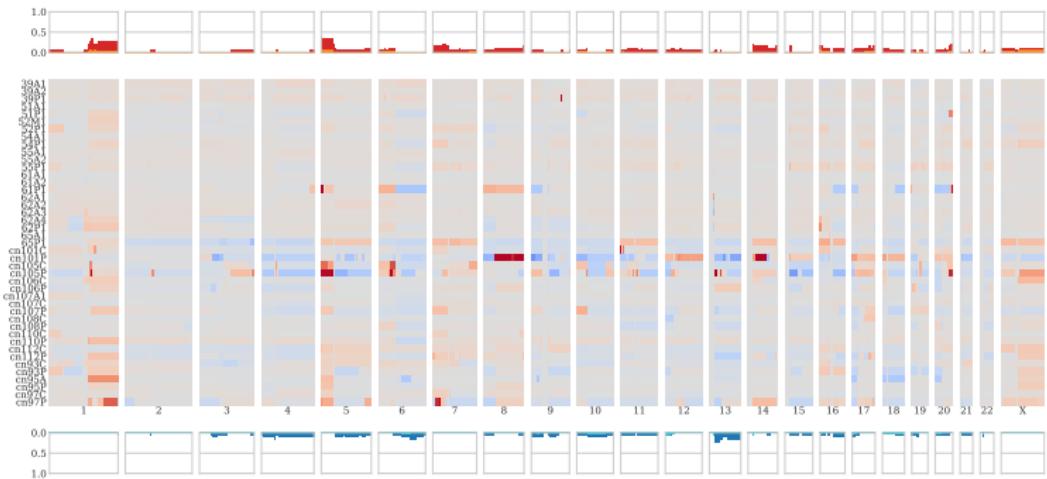


Figure: CNV Plot with LUAD Patients

# LUSC vs. LUAD in CNV Plot

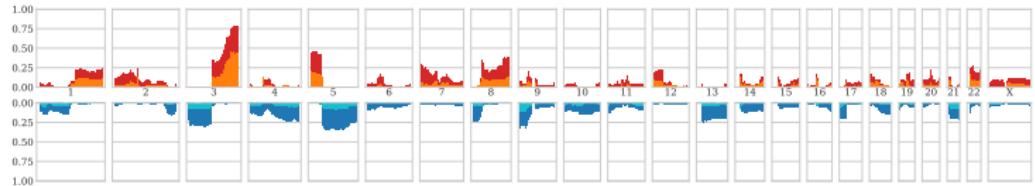


Figure: Simple CNV Plot with LUSC Patients

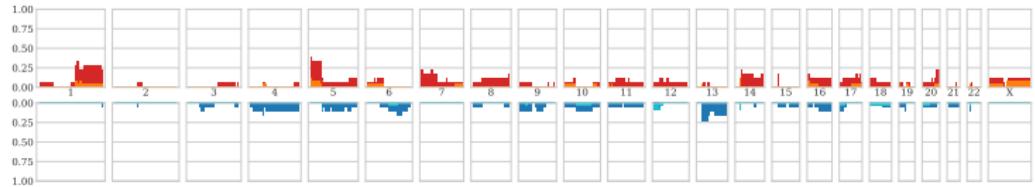


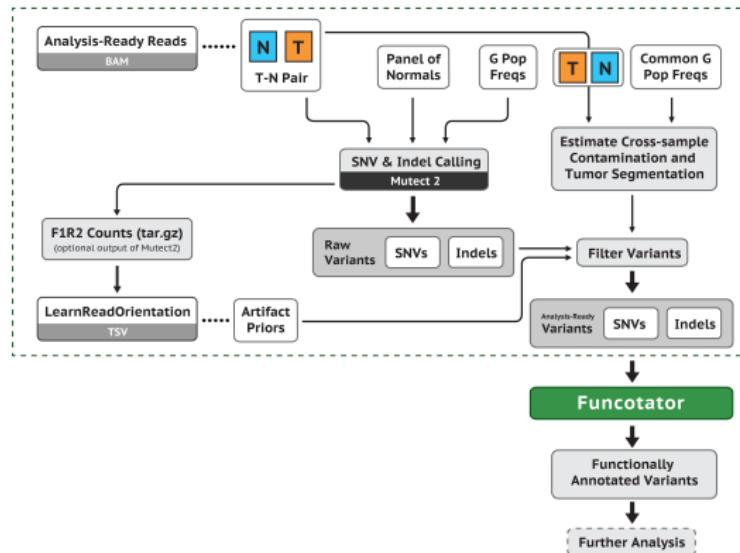
Figure: Simple CNV Plot with LUAD Patients

# Findings in Sequenza

## 4. Results

### 4.3. 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)

# 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

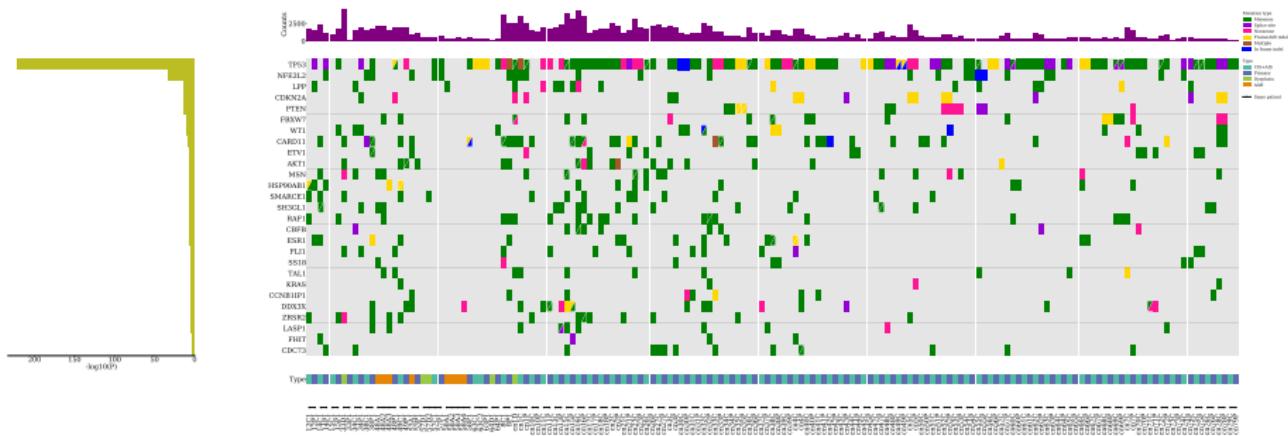


Figure: CoMut Plot with LUSC Patients

# Somatic Variant in LUAD

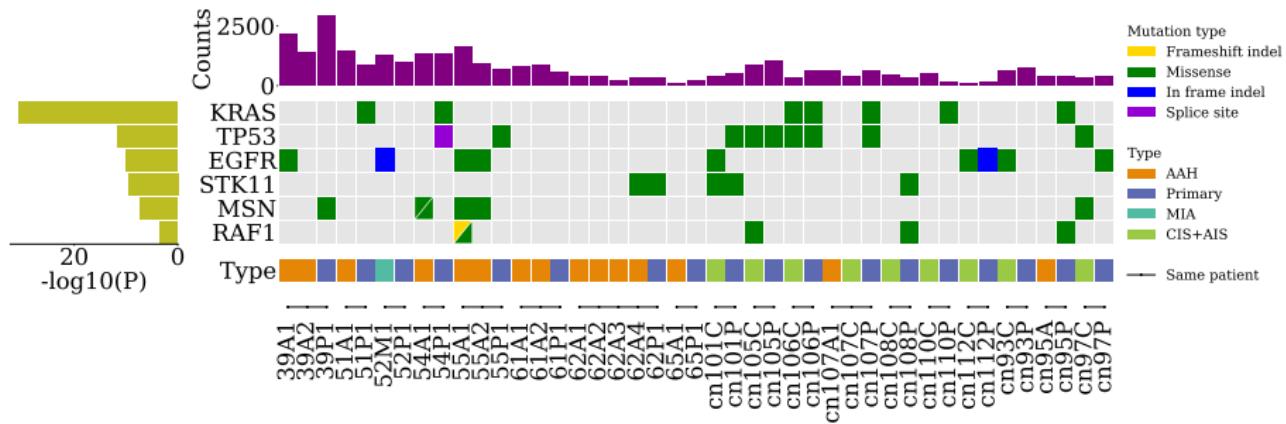


Figure: CoMut Plot with LUAD Patients

# Findings in SNVs Analysis

## 4. Results

### 4.4. VAF Analysis

# VAF?

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

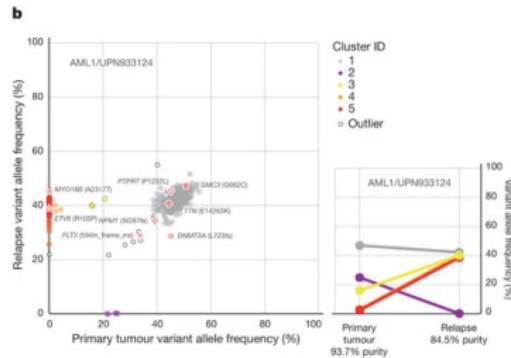


Figure: VAF distribution of validated mutations (Ding et al., 2012)

# VAF Plots I

# PyClone?

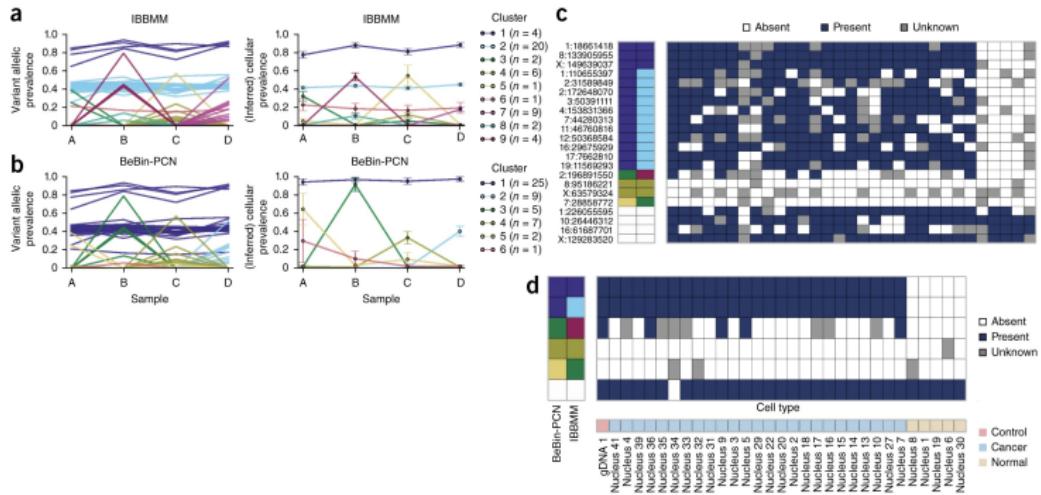


Figure: Analysis of multiple samples by PyClone (Roth et al., 2014)

# PyClone Plots I

# Findings in VAF Analysis

## 4. Results

### 4.5. Tumor Evolution Trajectories Analysis

# Revolver?

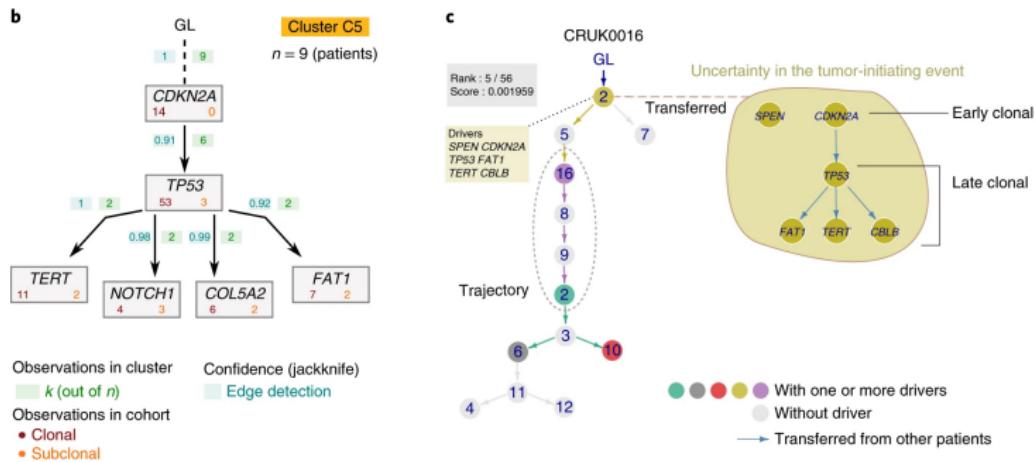


Figure: Repeated Evolutionary Trajectories (Caravagna et al., 2018)

# Findings in Tumor Evolution Trajectories Analysis

## 4. Results

### 4.6. Differences in Gene Expression Levels

# RSEM?

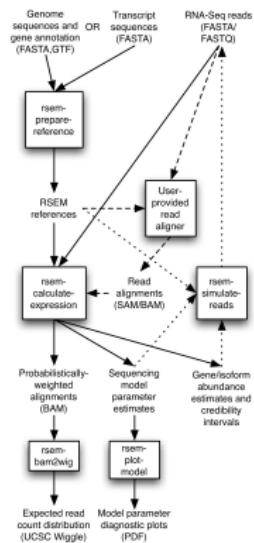


Figure: RSEM workflow (Li & Dewey, 2011)

# DESeq2?

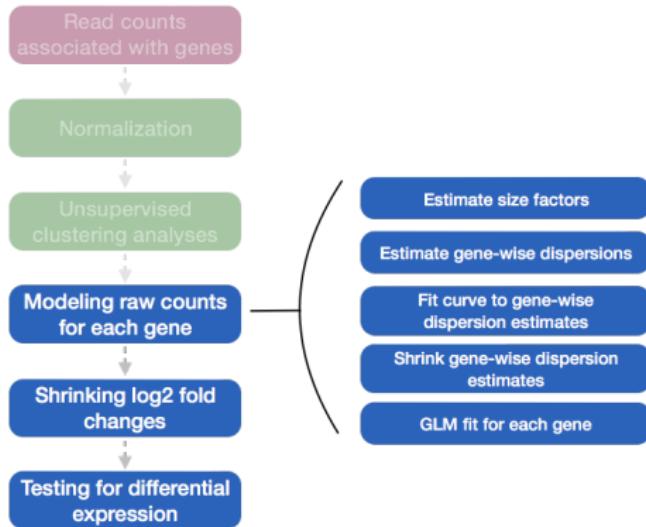


Figure: DESeq2 workflow (Love, Huber, & Anders, 2014)

# 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?

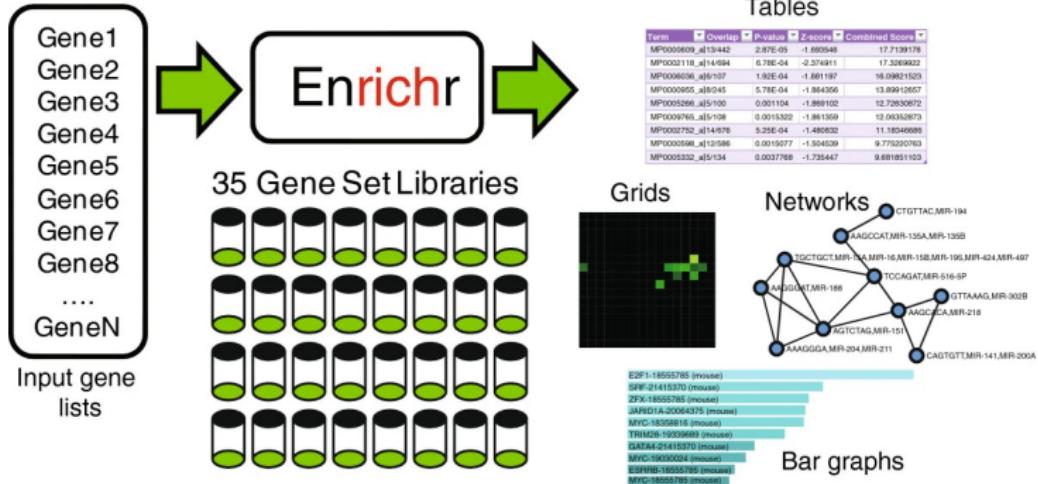


Figure: Enrichr workflow (Chen et al., 2013; Kuleshov et al., 2016)

# Gene-set Library

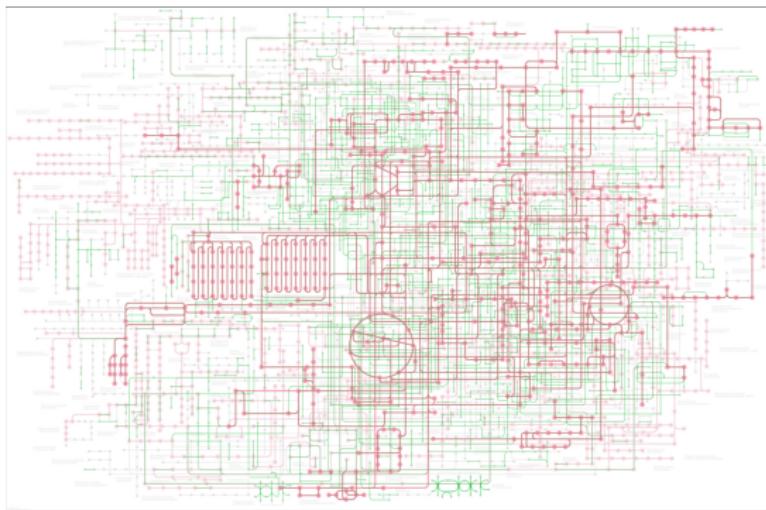


Figure: The global map of metabolic pathways by KEGG (Kanehisa et al., 2021)

KEGG

KEGG 2021 Human

# WTS Data Composition I

Table: Number of WTS samples

Cancer Subtype	Stage	Number of Samples
SQC (n=89)	Normal	17
	Dysplasia	2
	CIS	33
	Primary	35
ADC (n=30)	Normal	12
	AAH	1
	AIS	9
	MIA	0
	Primary	8

# WTS Data Composition II

Table: Number of WTS LUSC samples

Recurrence?	Stage	Number of Samples
Recurrence (n=13)	Normal	1
	Dysplasia	1
	CIS	5
	Primary	6
Non-recurrence (n=74)	Normal	16
	Dysplasia	1
	CIS	28
	Primary	29

# WTS Data Composition III

Table: Number of WTS LUAD samples

Recurrence?	Stage	Number of samples
Recurrence (n=4)	Normal	1
	AAH	0
	AIS	2
	MIA	0
	Primary	1
Non-recurrence (n=26)	Normal	11
	AAH	1
	AIS	7
	MIA	0
	Primary	7

## 4. Results

### 4.6. Differences in Gene Expression Levels

#### 4.6.1. Comparing cancer stage in LUSC

# DEG List in LUSC

Table: Up-regulated DEG in LUSC

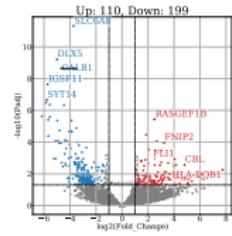
gene	log2FoldChange	pvalue	padj
AKR1C1	6.18e+00	5.14e-26	5.01e-23
AKR1C2	6.06e+00	1.19e-22	5.04e-20
CYP4F11	5.58e+00	1.51e-20	4.36e-18

Table: Down-regulated DEG in LUSC

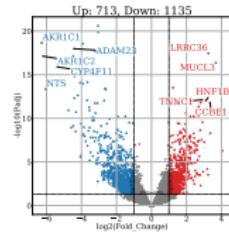
gene	log2FoldChange	pvalue	padj
SFTPC	-5.85e+00	9.16e-21	2.83e-18
FAM107A	-4.62e+00	2.27e-33	9.60e-30
LRRC36	-4.53e+00	5.49e-36	3.48e-32

# DEG Volcano Plots in LUSC

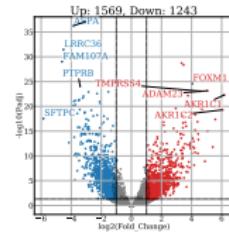
Normal → Dysplasia → CIS → Primary (LUSC)



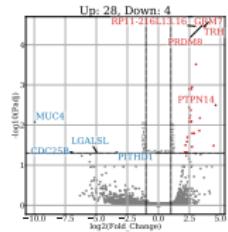
(a) Normal-Dysplasia



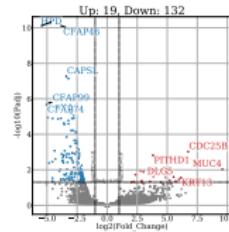
(b) Normal-CIS



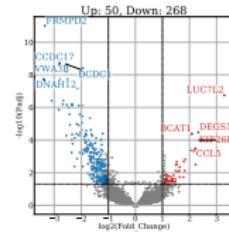
(c) Normal-Primary



(d) Dysplasia-CIS



(e) Dysplasia-Primary

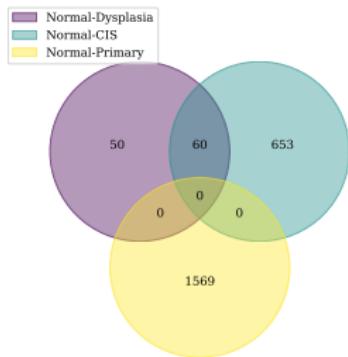


(f) CIS-Primary

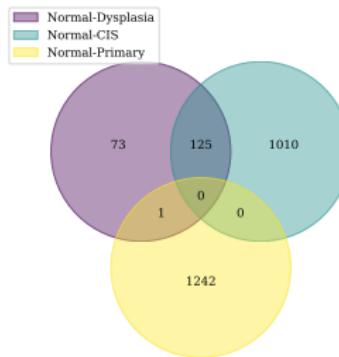
Figure: DEG Volcano Plots in LUSC

# DEG Venn Diagram in LUSC

Normal → Dysplasia → CIS → Primary (LUSC)



(a) Up-regulated



(b) 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,...	6.72e-03
Lysosome	ASAHI,LAPTM5,CTSH,...	6.72e-03
Phagosome	OLR1,HLA-DOA,TLR4,...	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,...	7.22e-08
Malaria	CSF3,HGF,ITGB2,...	1.16e-06
Cell adhesion molecules	NLGN3,SELPLG,CADM1,...	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,...	9.34e-06
Drug metabolism	GSTM4,GSTM3,GSTM2,...	9.06e-05
Cell cycle	CDKN2A,PLK1,BUB1B,...	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,...	1.53e-04
Glutathione metabolism	GSTM4,GSTM3,G6PD,...	1.53e-04
DNA replication	FEN1,RNASEH2A,RFC4,...	1.72e-04

Table: Down-regulated Pathways on Normal vs. Primary

Term name	Overlapping genes...	Adjusted p-value
Hematopoietic cell lineage	CSF1R,CSF3,CSF3R,...	7.33e-09
Malaria	IL10,CSF3,CR1,...	7.33e-09
Hypertrophic cardiomyopathy	LAMA2,ITGB3,CACNA1D,...	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

### 4.6. Differences in Gene Expression Levels

#### 4.6.2. Comparing cancer stage in LUAD

# DEG List in LUSC

Table: Up-regulated DEG in LUAD

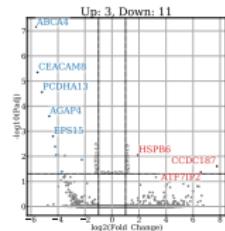
gene	log2FoldChange	pvalue	padj
ABCA4	4.95e+00	3.01e-12	2.58e-09
HMGA2	4.79e+00	8.06e-08	1.46e-05
KIF12	4.48e+00	1.33e-06	1.46e-04

Table: Down-regulated DEG in LUAD

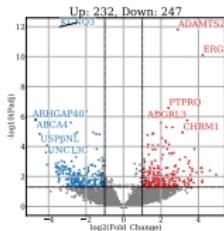
gene	log2FoldChange	pvalue	padj
SLC6A4	-6.20e+00	5.80e-10	2.36e-07
IL1RL1	-4.20e+00	7.47e-06	5.82e-04
RNF185	-4.06e+00	4.75e-05	2.45e-03

# DEG Volcano Plots in LUAD

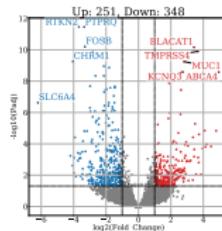
Normal → AAH → AIS → Primary (LUAD)



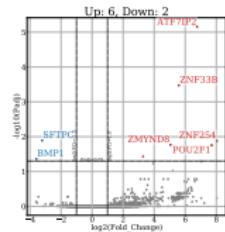
(a) Normal-AAH



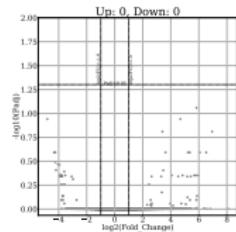
(b) Normal-AIS



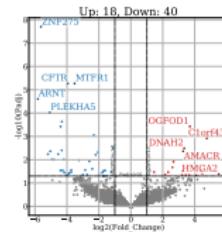
(c) Normal-Primary



(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) 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,...	2.49e-02
Cell adhesion molecules	CDH5,CLDN5,NLGN1,...	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,...	1.38e-04
ECM-receptor interaction	TNXB, VWF, COL4A2,...	3.58e-04
Calcium signaling pathway	MCOLN3, CHRM1, NOS2,...	4.03e-04

# Finding in Comparing cancer stage in LUAD

## 4. Results

### 4.6. Differences in Gene Expression Levels

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

# LUSC Data Composition

Table: Number of WTS LUSC samples

Recurrence?	Stage	Number of Samples
Recurrence (n=13)	Normal	1
	Dysplasia	1
	CIS	5
	Primary	6
Non-recurrence (n=74)	Normal	16
	Dysplasia	1
	CIS	28
	Primary	29

## Normal samples

In order to compare with Normal stage, merging Normal samples.  
∴ Insufficient number of Normal samples in Recur.

# DEG List for Recur in CIS of LUSC

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

gene	log2FoldChange	pvalue	padj
ATAD5	3.62e+00	1.97e-07	6.43e-04
RGPD2	3.28e+00	1.28e-06	3.33e-03
KCNN3	3.25e+00	1.37e-07	6.43e-04

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

gene	log2FoldChange	pvalue	padj
ARL13B	-4.64e+00	1.05e-05	1.36e-02
PARP11	-4.18e+00	1.60e-07	6.43e-04
ADCK2	-3.57e+00	6.41e-05	4.91e-02

# DEG List for Recur in Primary of LUSC

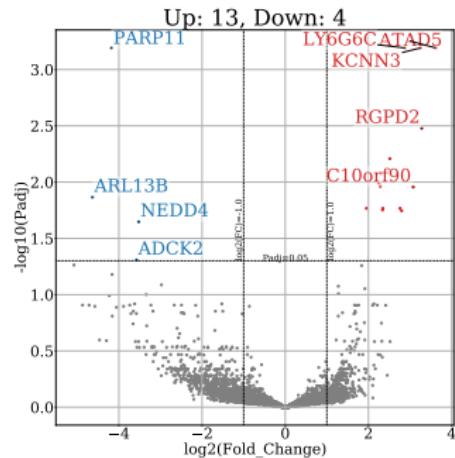
Table: Up-regulated DEG for Recur in Primary of LUSC

gene	log2FoldChange	pvalue	padj
GPC5	2.88e+00	1.58e-06	9.87e-03
AR	2.54e+00	5.21e-05	5.00e-02
COPE	2.30e+00	1.43e-06	9.87e-03

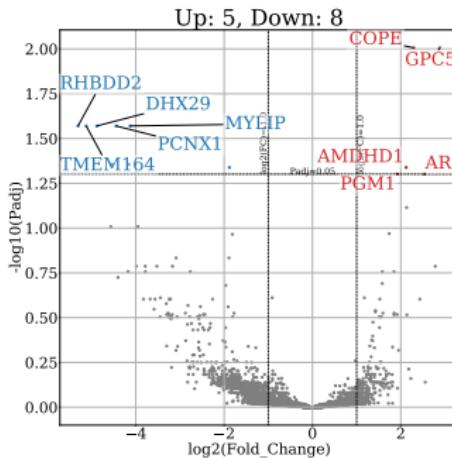
Table: Down-regulated DEG for Recur in Primary of LUSC

gene	log2FoldChange	pvalue	padj
RHBDD2	-5.31e+00	1.08e-05	2.69e-02
TMEM164	-5.12e+00	1.51e-05	2.69e-02
DHX29	-4.88e+00	9.47e-06	2.69e-02

# DEG Volcano Plots Recur vs. Non-recur in LUSC



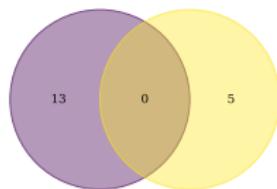
(a) CIS



(b) Primary

Figure: DEG Volcano Plots Recur vs. Non-recur

# DEG Venn Diagram Recur vs. Non-recur with CIS in LUSC



(a) Up-regulated



(b) Down-regulated

Figure: DEG Venn Diagram Recur vs. Non-recur

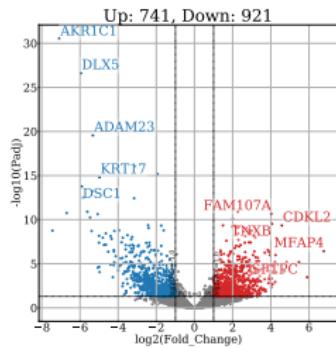
# Finding in Comparing Recur vs. Non-recur in LUSC

## 4. Results

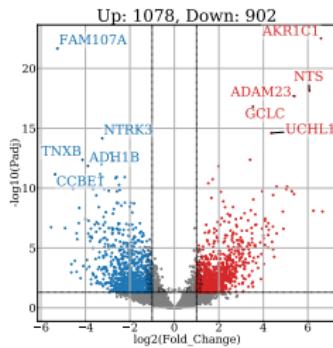
### 4.6. Differences in Gene Expression Levels

#### 4.6.4. Within Recur in LUSC

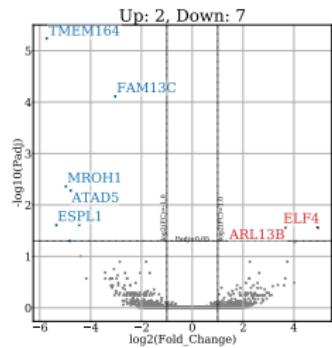
# DEG Volcano Plots with Recur in LUSC



(a) Normal-CIS



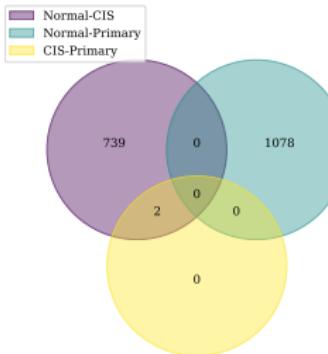
(b) Normal-Primary



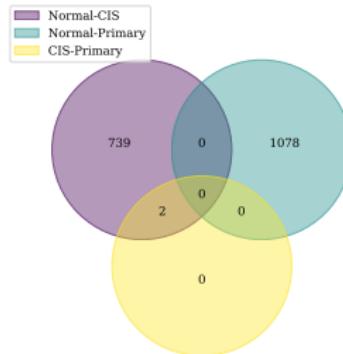
(c) CIS-Primary

Figure: DEG Volcano Plots with Recur samples in LUSC

# DEG Venn Diagram with Recur in LUSC



(a) Up-regulated



(b) 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,...	1.87e-05
Cell adhesion molecules	SELPLG,CADM1,SDC3,...	1.87e-05
Hypertrophic cardiomyopathy	EDN1,ACE,TNNC1,...	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,...	2.11e-05
Alzheimer disease	COX7B,NDUFA12,NDUFB5,...	2.11e-05
Huntington disease	DCTN5,COX7B,NDUFA12,...	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,...	1.90e-05
RNA transport	EIF4A2,NUP205,NUP62,...	2.66e-05
Drug metabolism	GSTM4,GSTM3,GSTM2,...	2.66e-05

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

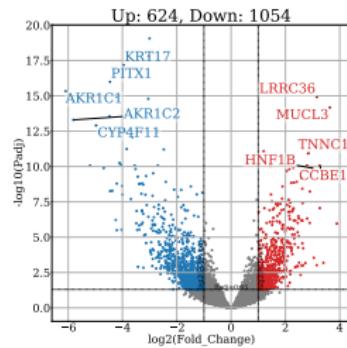
Term name	Overlapping genes...	Adjusted p-value
Dilated cardiomyopathy	LAMA2,ITGA3,TNNC1,...	2.19e-06
Hypertrophic cardiomyopathy	LAMA2,ITGA3,TNNC1,...	2.19e-06
Arrhythmogenic right ventricular cardiomyopathy	TCF7L2,LAMA2,ACTN2,...	4.12e-06

## 4. Results

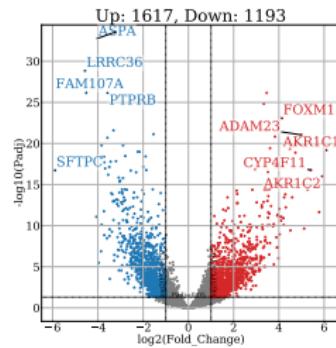
### 4.6. Differences in Gene Expression Levels

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

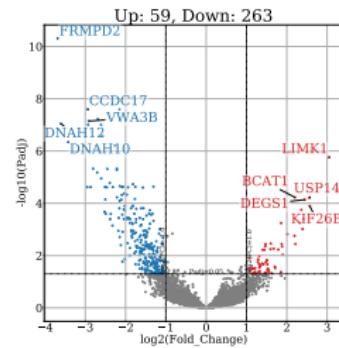
# DEG Volcano Plots with Non-recr in LUSC



(a) Normal-CIS



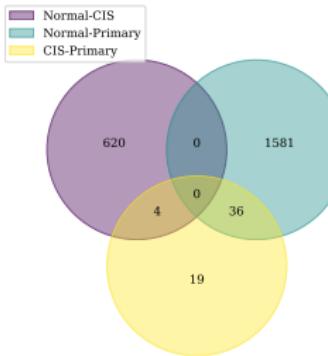
(b) Normal-Primary



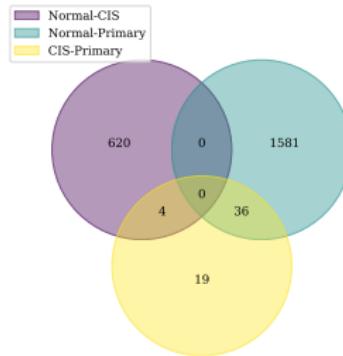
(c) CIS-Primary

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

# DEG Venn Diagram with Non-recr in LUSC



(a) Up-regulated



(b) Down-regulated

Figure: DEG Venn Diagram with Non-recr 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,...	6.53e-08
Hematopoietic cell lineage	CSF1R,CSF3,MME,...	2.01e-07
Hypertrophic cardiomyopathy	EDN1,ACE,LAMA2,...	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,...	9.67e-05
Drug metabolism	GSTM4,GSTM3,RRM1,...	1.18e-04
Cell cycle	GADD45A,CDKN2A,PLK1,...	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,...	3.04e-06
DNA replication	FEN1,RNASEH2A,RFC4,...	6.47e-06
Homologous recombination	BLM,RPA1,PALB2,...	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,...	6.65e-10
Malaria	IL10,CSF3,CR1,...	3.57e-09
Hypertrophic cardiomyopathy	LAMA2,ITGB3,CACNA1D,...	5.12e-09

## 4. Results

### 4.6. Differences in Gene Expression Levels

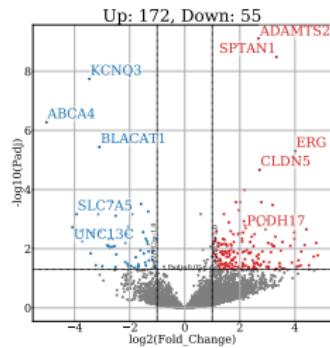
#### 4.6.6. Within Non-recur in LUAD

# LUAD Data Composition

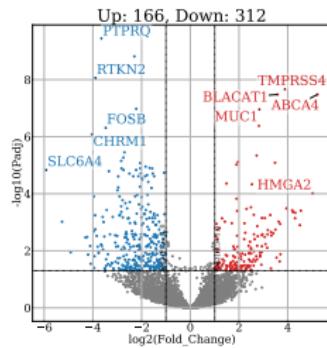
Table: Number of WTS LUAD samples

Recurrence?	Stage	Number of samples
Recurrence (n=4)	Normal	1
	AAH	0
	AIS	2
	MIA	0
	Primary	1
Non-recurrence (n=26)	Normal	11
	AAH	1
	AIS	7
	MIA	0
	Primary	7

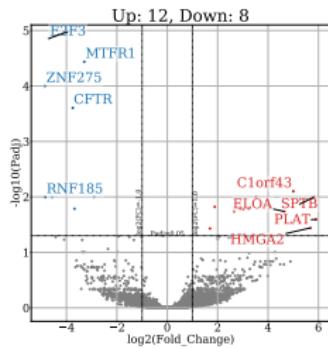
# DEG Volcano Plots with Non-recr in LUAD



(a) Normal-AIS



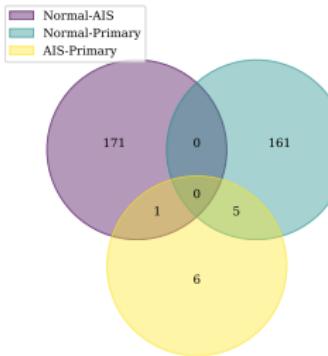
(b) Normal-Primary



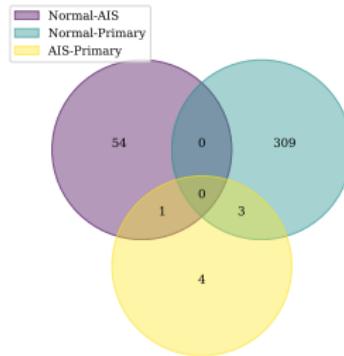
(c) AIS-Primary

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

# DEG Venn Diagram with Non-recr in LUAD



(a) Up-regulated



(b) Down-regulated

Figure: DEG Venn Diagram with Non-recr 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,...	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

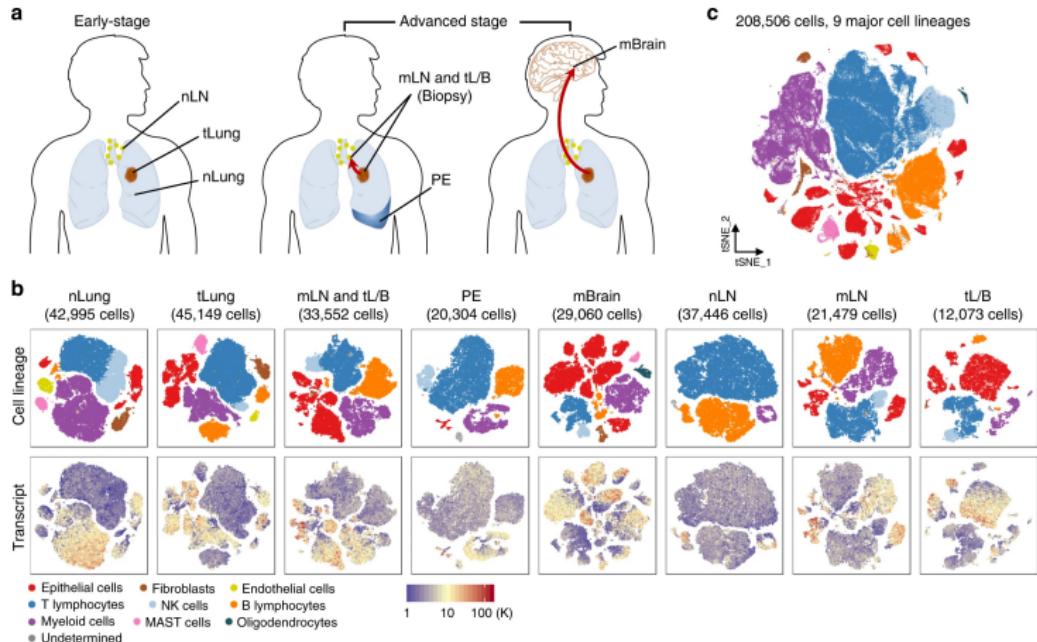
Term name	Overlapping genes...	Adjusted p-value
ECM-receptor interaction	TNXB,VWF,COL4A2,...	2.05e-03
Vascular smooth muscle contraction	PPP1R14A,EDN1,AGTR1,...	4.98e-03
Calcium signaling pathway	MCOLN3,CHRM1,NOS2,...	7.82e-03

# Findings in DEG Analysis

## 4. Results

### 4.7. 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?

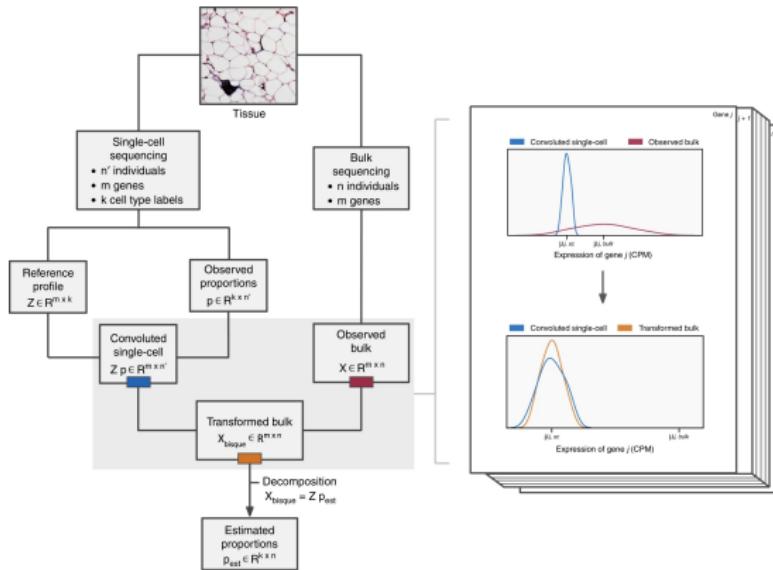


Figure: Workflow for BisqueRNA (Jew et al., 2020)

# Cluster Plot in LUSC

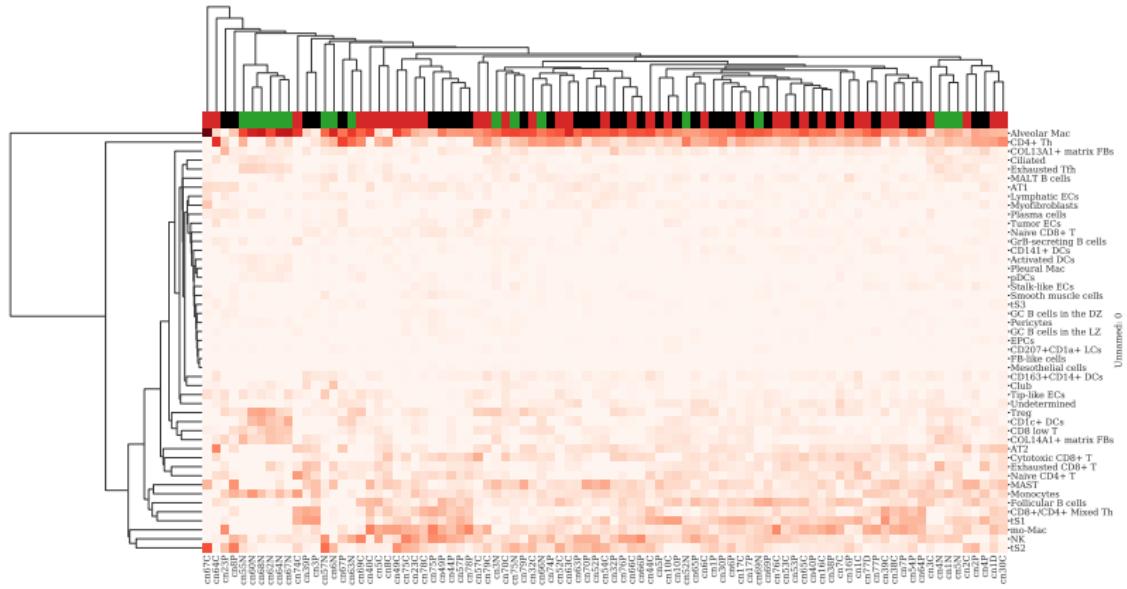


Figure: Cluster Plot in LUSC

# Violin Plots in LUSC I

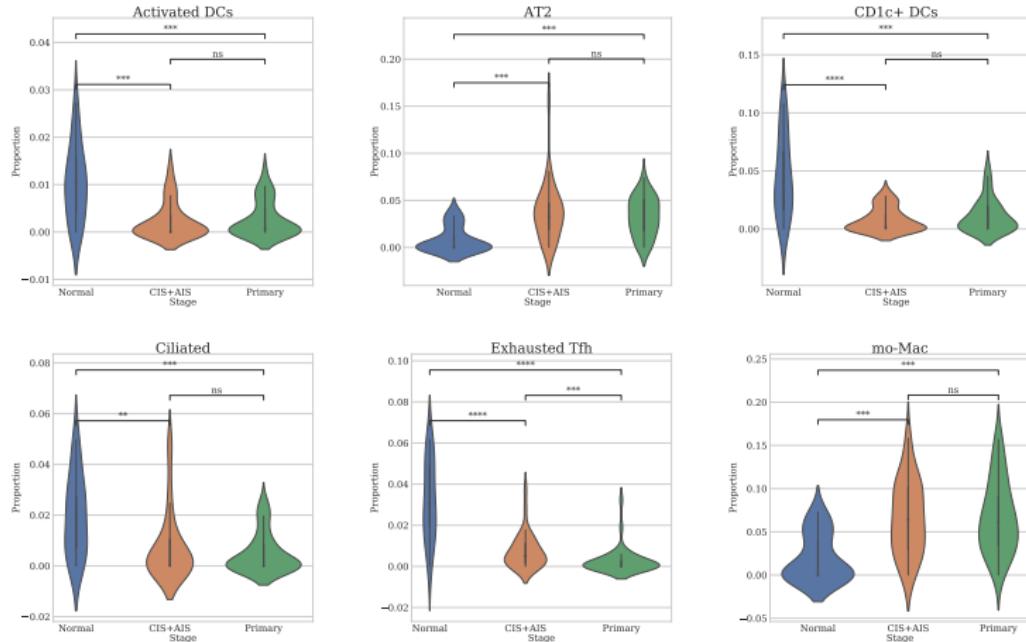


Figure: Violin Plots in LUSC

# Violin Plots in LUSC II

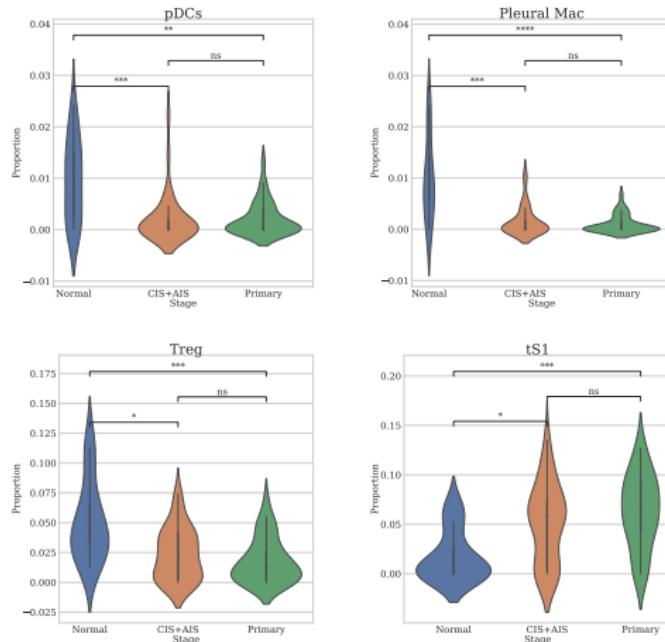


Figure: Violin Plots in LUSC

# Cluster Plot in LUAD

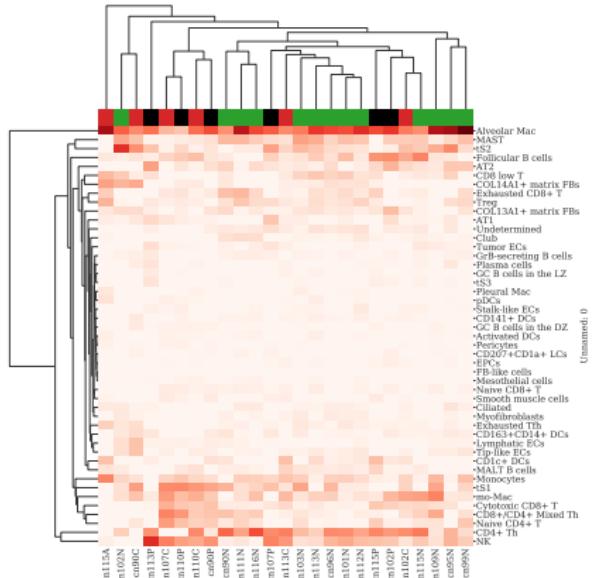


Figure: Cluster Plot in LUAD

# Violin Plots in LUAD

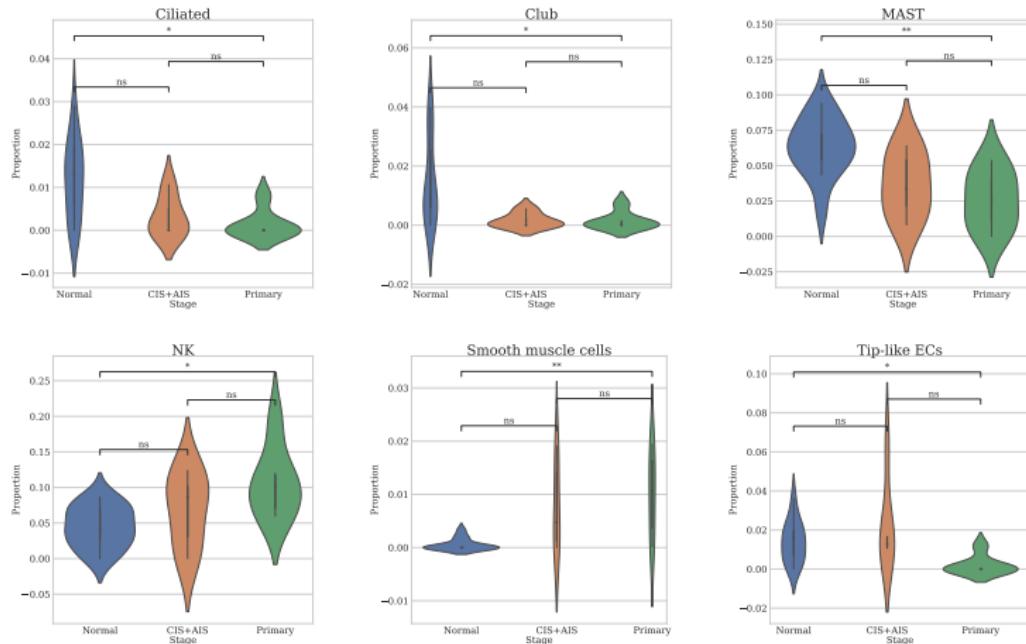


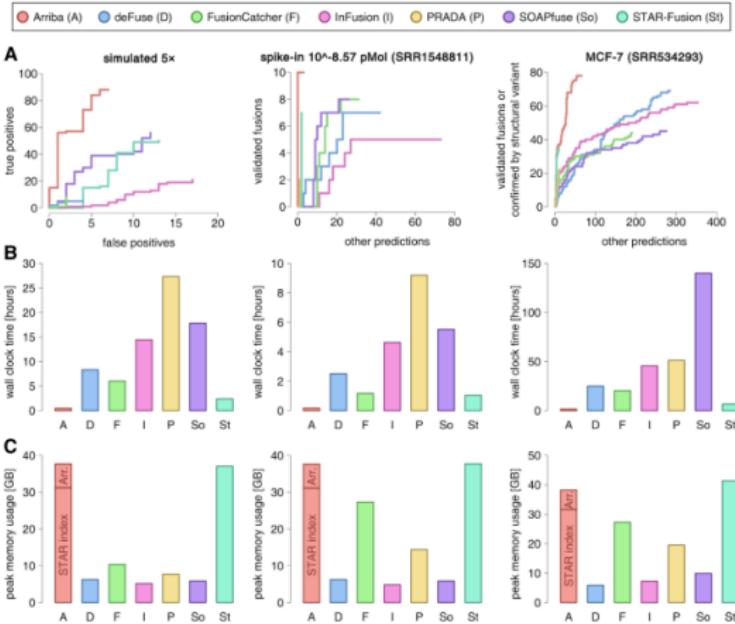
Figure: Violin Plots in LUAD

# Findings in Bulk Cell Deconvolution

## 4. Results

### 4.8. Discovery of Gene Fusion

# Arriba?



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

# Findings in Gene Fusion Discovery

## 5. Discussion

## 6. References

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