

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

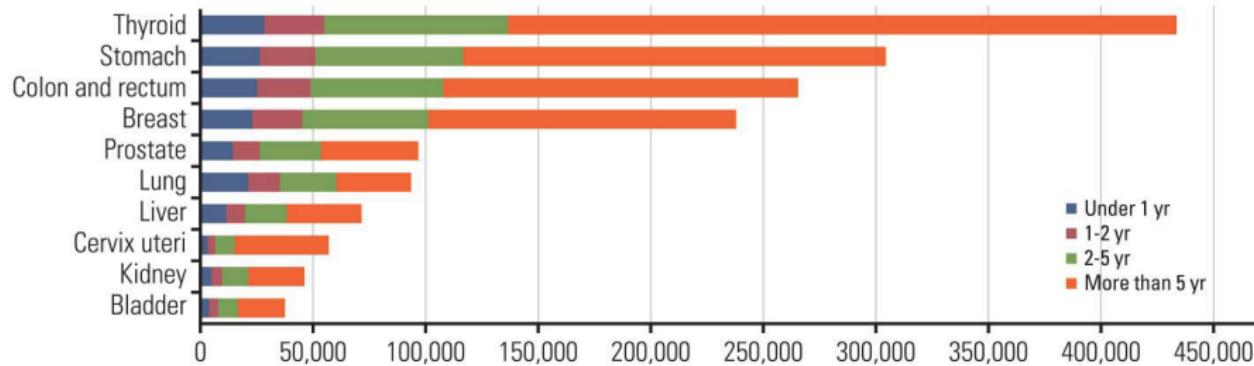


Figure: Common cancer survival rates (S. Hong et al., 2021)

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. Non-small cell lung cancer

Non-small cell lung cancer (NSCLC)

1. Introduction

1.3. LUAD

LUAD

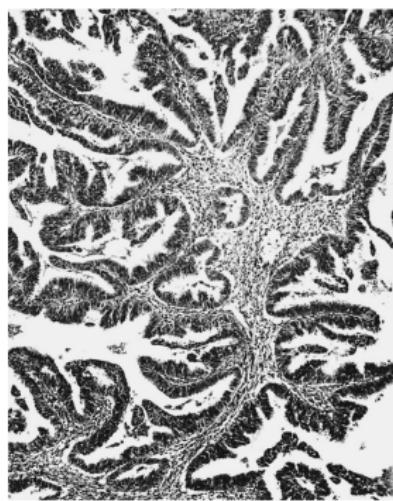
1. Introduction

1.4. LUSC

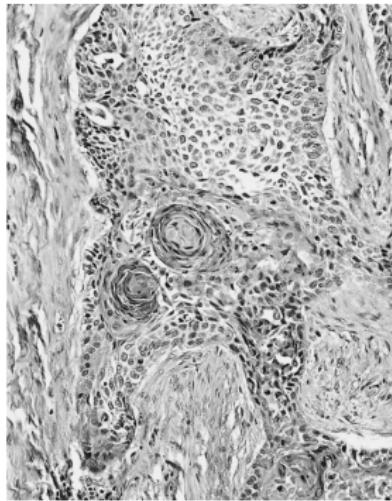
1. Introduction

1.5. LUAD vs. LUSC

LUAD vs. LUSC I



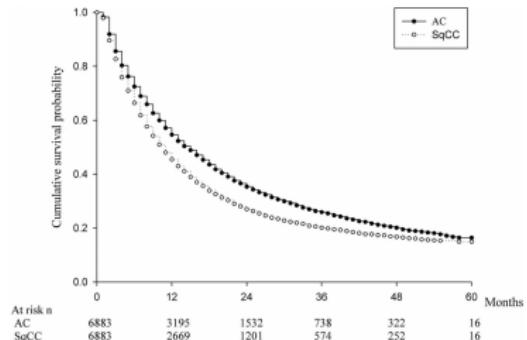
(a) LUAD



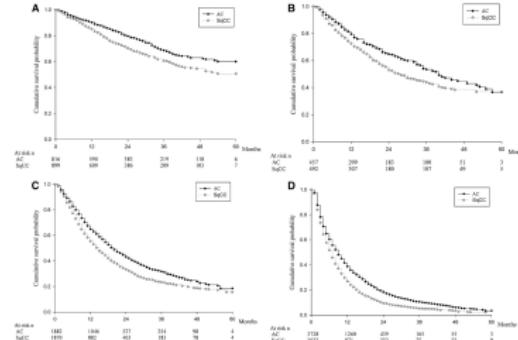
(b) LUSC

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

LUAD vs. LUSC II



(a) All patients



(b) By cancer stages

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

Findings

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

1. Introduction

1.6. Study Objectives

Study Objectives

Find different mutations

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

Pathway examine

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

Ultra-deep sequencing

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

- from blood
- from urine
- from bronchus

2. Materials

Lung Cancer Data

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

2. Materials

2.1. WES Data

WES Data Composition

Table: Number of WES samples

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

WES Data Composition with Recurrence I

Table: LUSC WES Data with Recurrence

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

WES Data Composition with Recurrence II

Table: LUAD WES Data with Recurrence

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

WES Data Composition with Smoking I

Table: LUSC WES Data with Smoking

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

WES Data Composition with Smoking II

Table: LUAD WES Data with Smoking

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

2. Materials

2.2. WTS Data

WTS Data Composition

Table: Number of WTS samples

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

WTS Data Composition with Recurrence I

Table: LUSC WTS Data with Recurrence

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

WTS Data Composition with Recurrence II

Table: LUAD WTS Data with Recurrence

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

WTS Data Composition with Smoking I

Table: LUSC WTS Data with Smoking

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

WTS Data Composition with Smoking II

Table: LUAD WTS Data with Smoking

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

3. Methods

3. Methods

3.1. Workflows

Data pre-processing for variant discovery

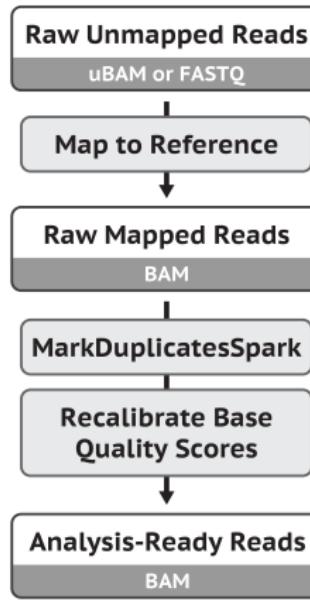


Figure: Data pre-processing for variant discovery (Van der Auwera et al., 2013; DePristo et al., 2011)

Somatic short variant discovery

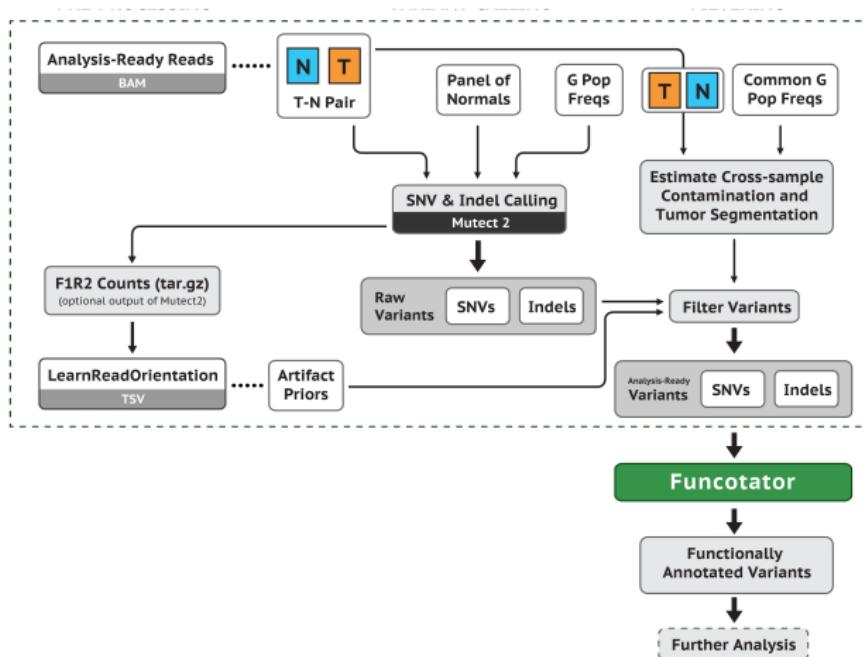


Figure: Somatic short variant (SNVs + Indels) discovery workflow (Van der Auwera et al., 2013; DePristo et al., 2011)

Germline short variant discovery

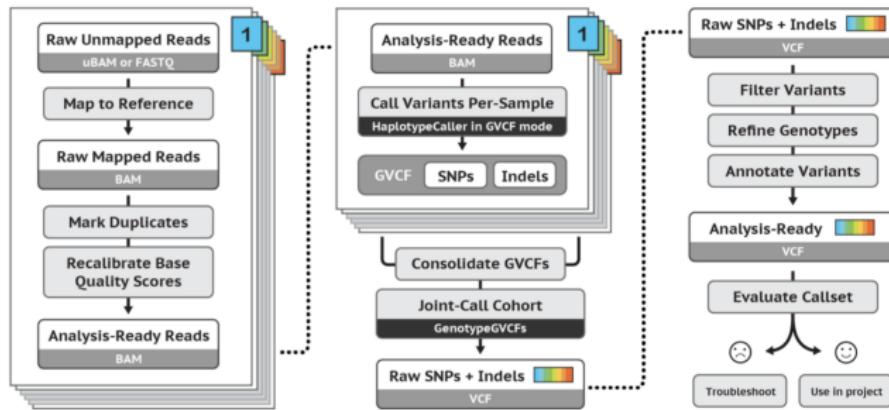


Figure: Germline short variant (SNVs + Indels) discovery workflow (Van der Auwera et al., 2013; DePristo et al., 2011)

RNA-seq short variant discovery

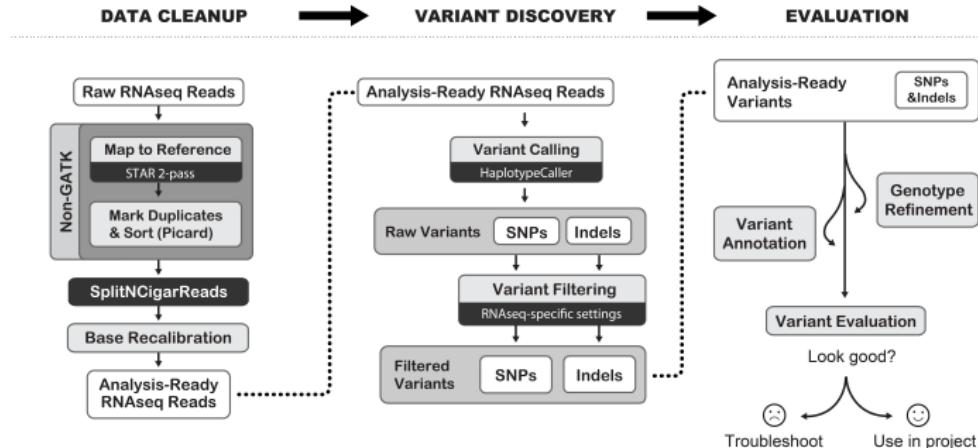


Figure: RNA-seq short variant (SNVs + Indels) discovery workflow (Van der Auwera et al., 2013; DePristo et al., 2011)

4. Results

4. Results

4.1. Quality Checks with FastQC

FastQC?

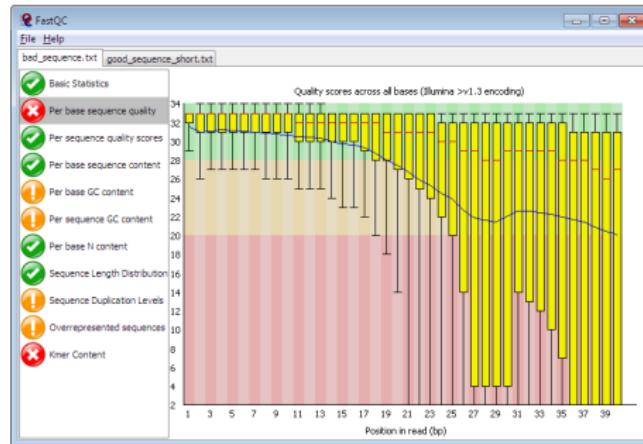


Figure: Example of FastQC Result (Andrews et al., 2012)

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

FastQC on WES

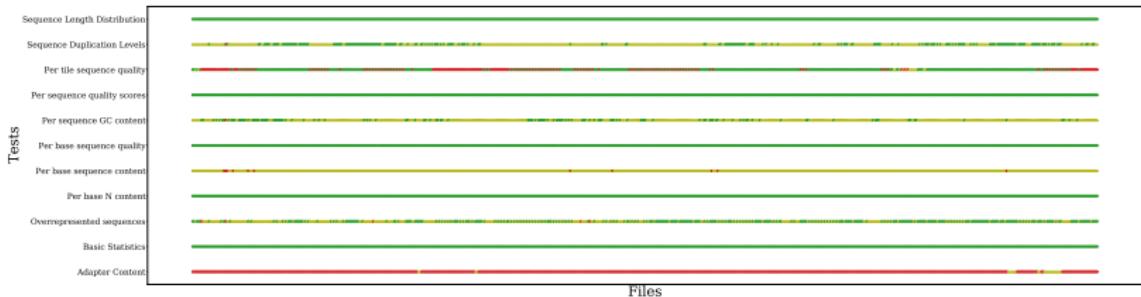
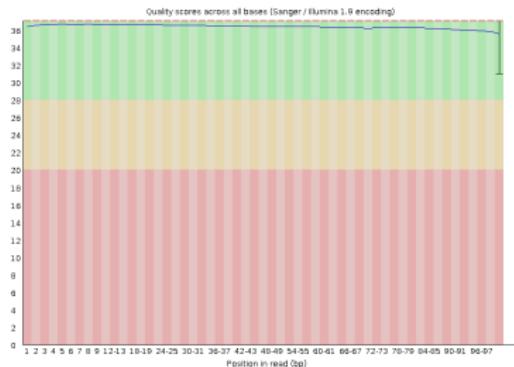


Figure: FastQC with WES data

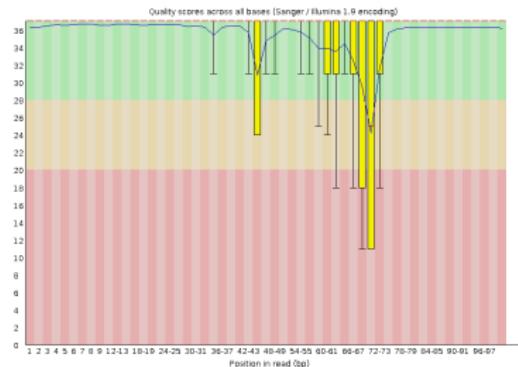
Failure on 33P1 sample

33P1 is excluded at further analysis.

Failure on 33P1 I



(a) 33N



(b) 33P1

Figure: Per Base Sequence Quality Results

Failure on 33P1 II

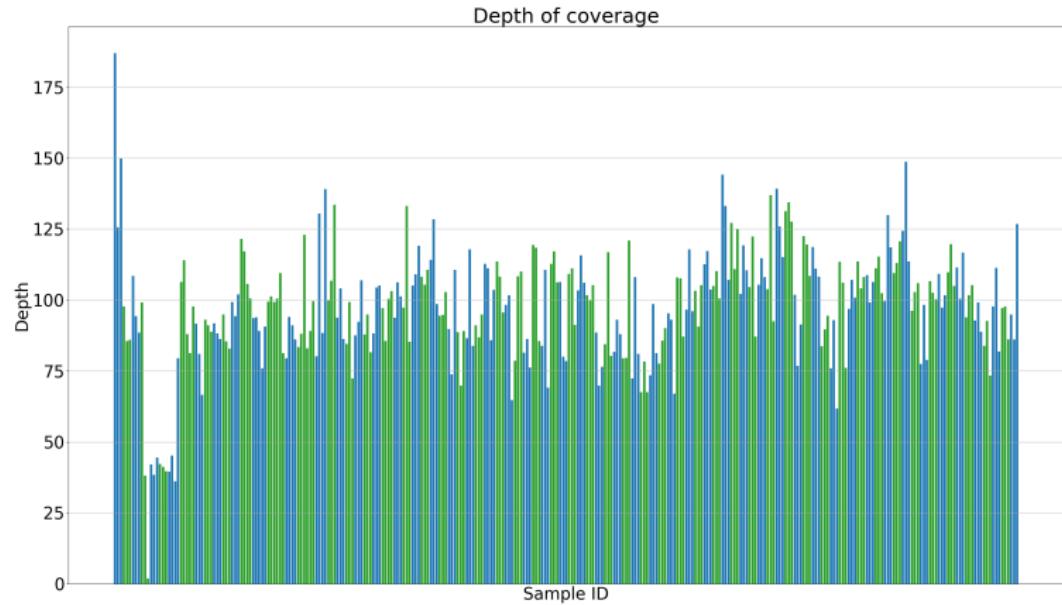


Figure: Coverage Depth Plot

FastQC on WTS

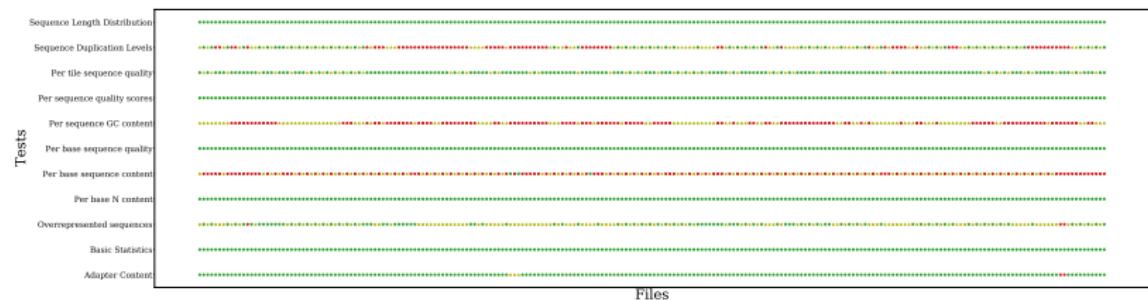


Figure: FastQC with WTS data

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?

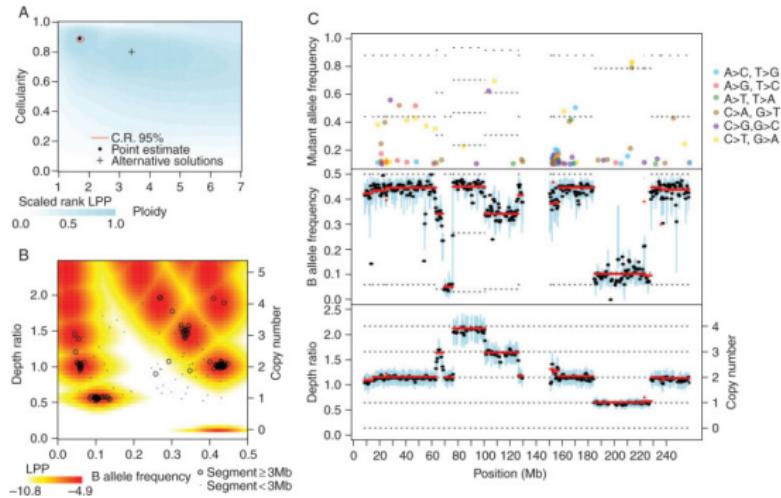
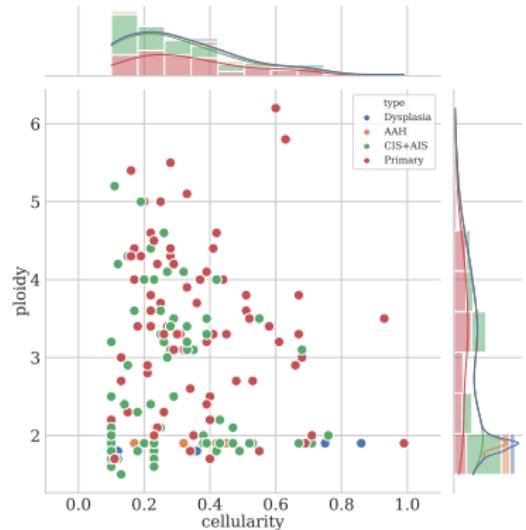
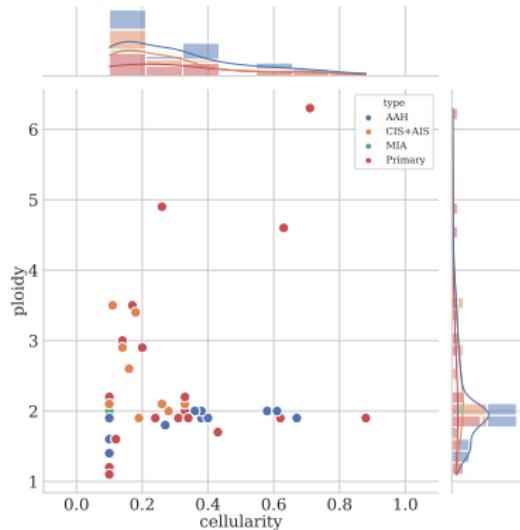


Figure: Representative Output of the Sequenza (Favero et al., 2015)

Cellularity & Ploidy on WES



(a) LUSC Samples



(b) LUAD Samples

Figure: Cellularity and Ploidy from Sequenza

LUSC in CNV Plot

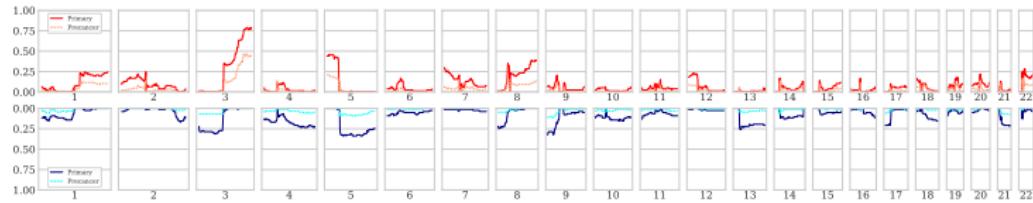


Figure: LUSC in CNV Plot

LUSC with Recurrence in CNV Plot

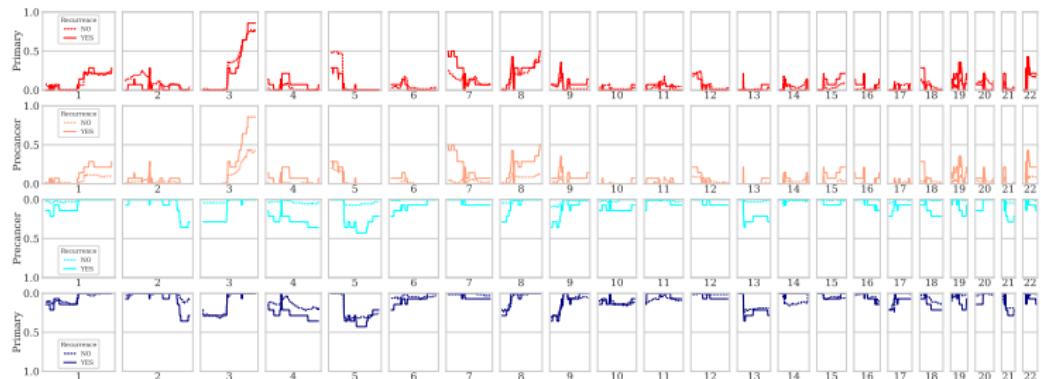


Figure: LUSC with Recurrence in CNV Plot

LUAD in CNV Plot

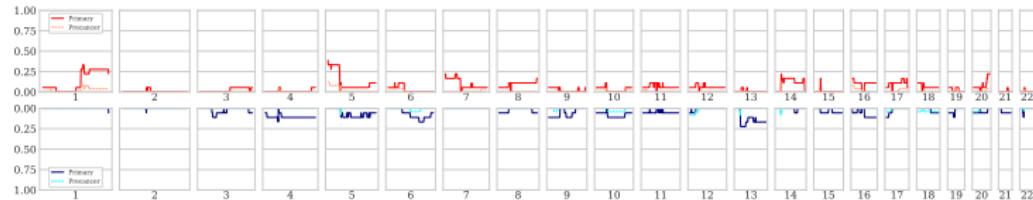


Figure: LUAD in CNV Plot

LUAD with Recurrence in CNV Plot

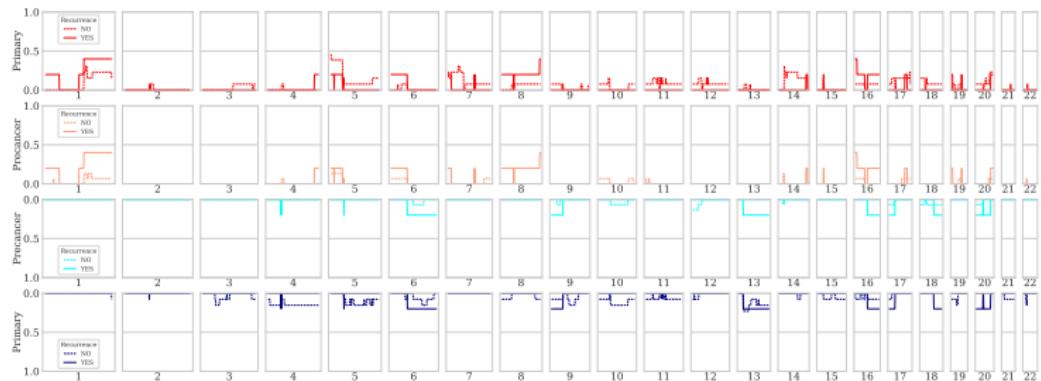


Figure: LUAD with Recurrence in CNV Plot

Findings in Sequenza

PureCN?

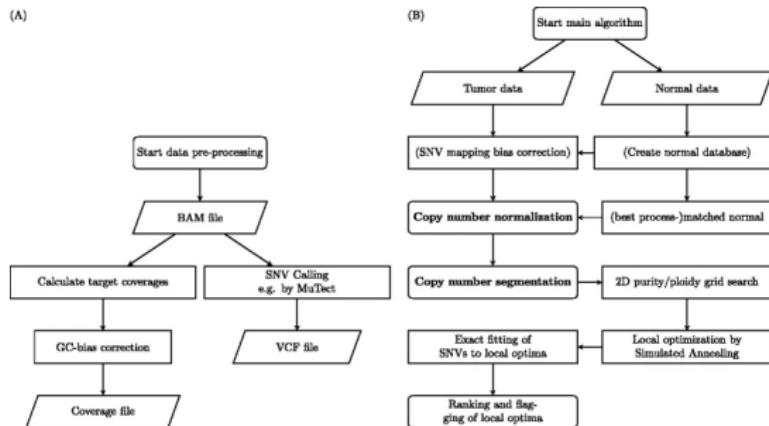


Figure: Flowchart of the PureCN data pre-processing pipeline (Riester et al., 2016)

Findings in PureCN

4. Results

4.4. SNVs Analysis

Mutect2?

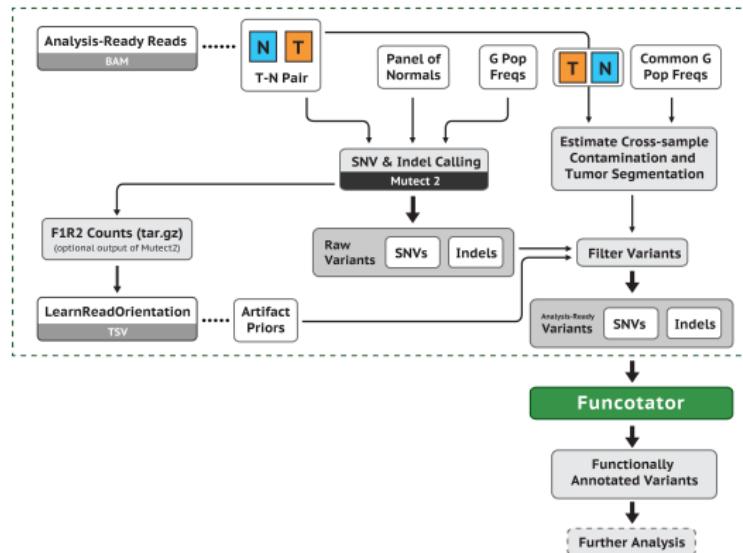
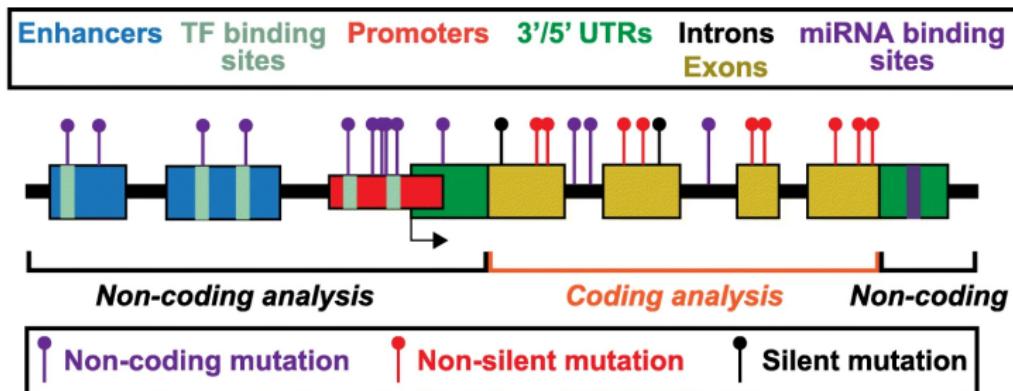


Figure: Somatic short variant discovery workflow (Van der Auwera et al., 2013; DePristo et al., 2011)

MutEnricher?



Analysis summary:

Inputs:

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

Analyses:

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

Outputs:

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

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

CoMut?

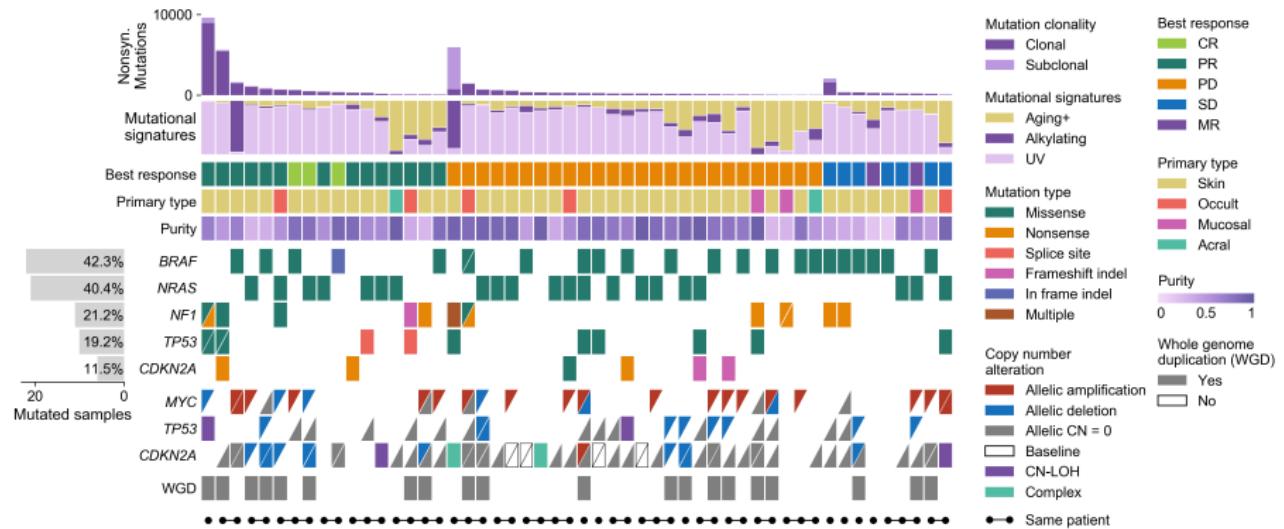


Figure: A comutation plot generated with CoMut (Crowdis et al., 2020)

Driver Gene Selection Strategy

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

Gene \in CGC Tier 1 set

Fisher FDR

Fisher FDR < 0.05

Fisher P-value

Fisher P-value < 0.05

Gene P-value

Gene P-value < 0.05

Somatic Variant in LUSC

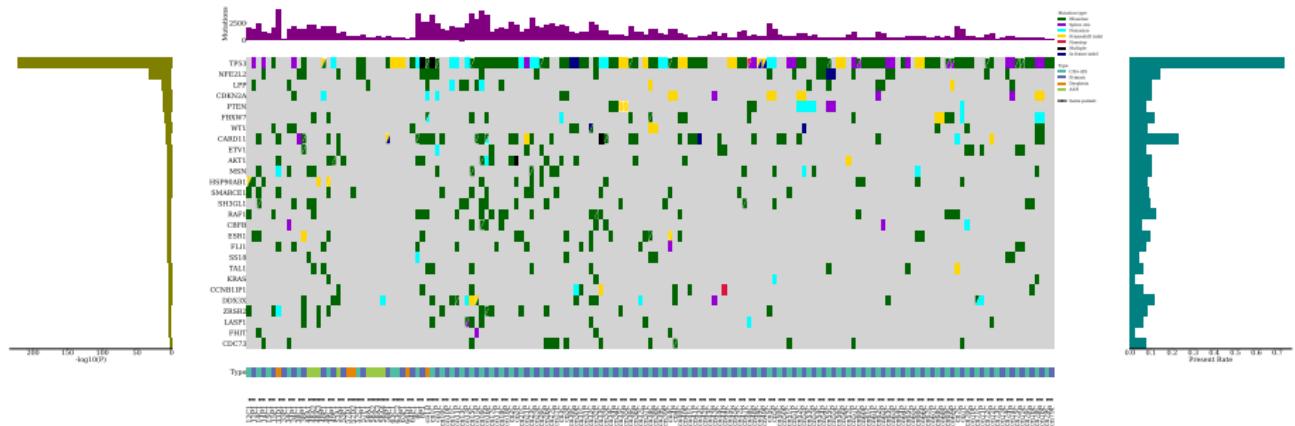


Figure: CoMut Plot with LUSC Patients

Somatic Variant in LUSC with Recurrence

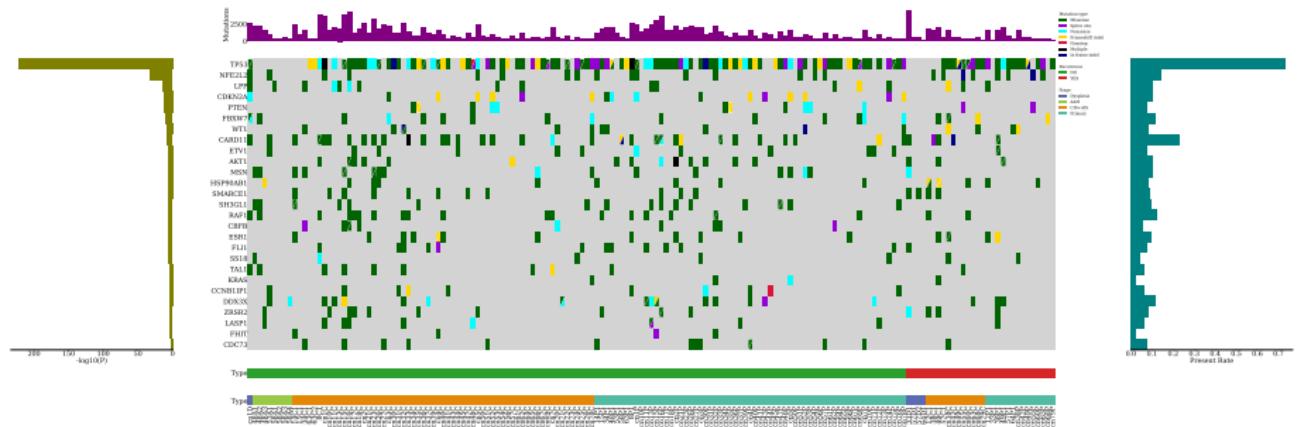


Figure: CoMut Plot in LUSC Patients with Recurrence

Somatic Variant in LUAD

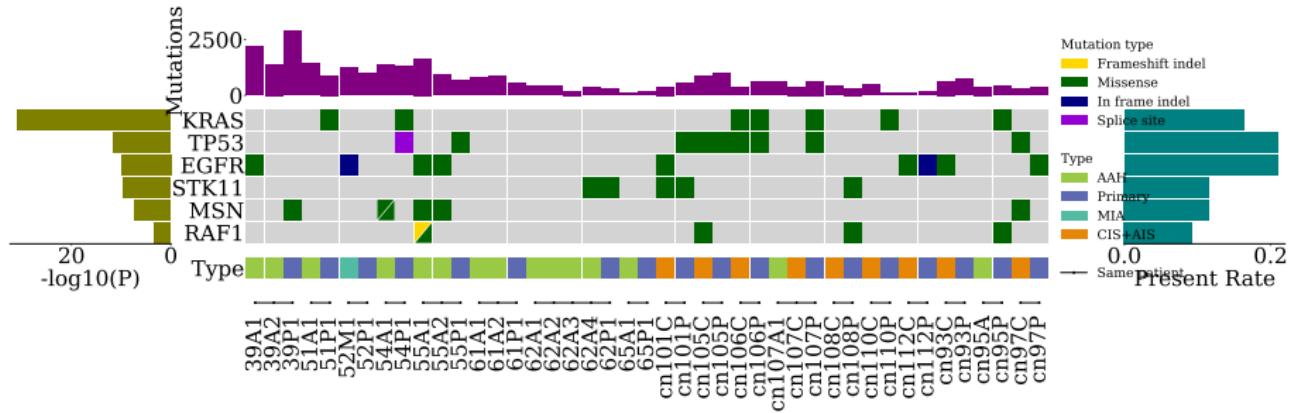


Figure: CoMut Plot with LUAD Patients

Somatic Variant in LUAD with Recurrence

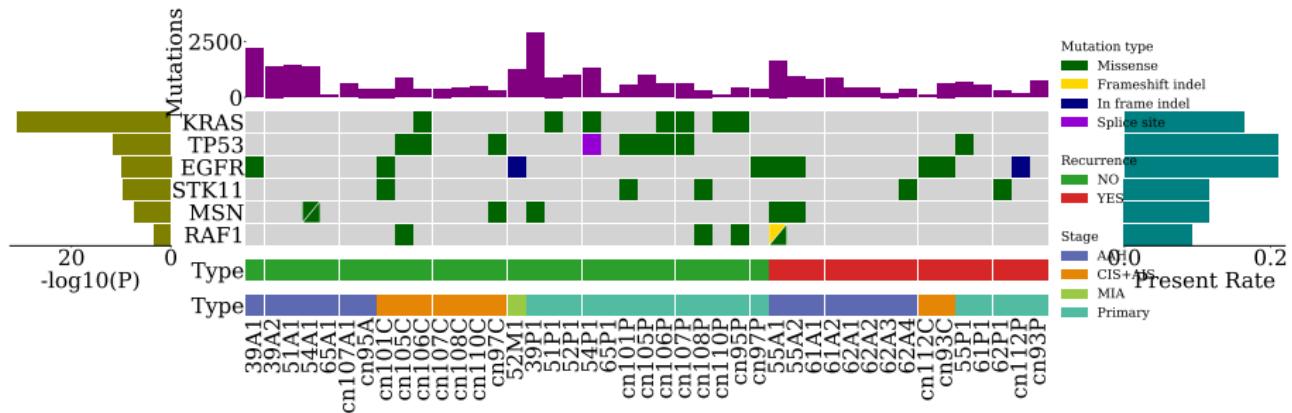


Figure: CoMut Plot in LUAD Patients with Recurrence

Findings in SNVs Analysis

4. Results

4.5. VAF Analysis

VAF?

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

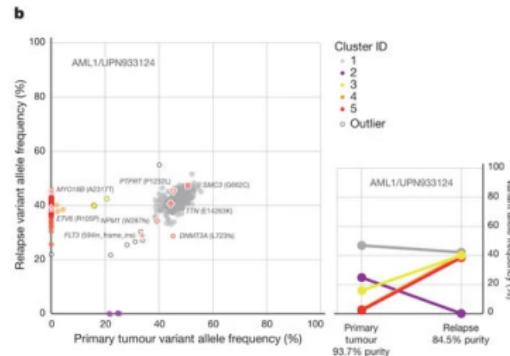
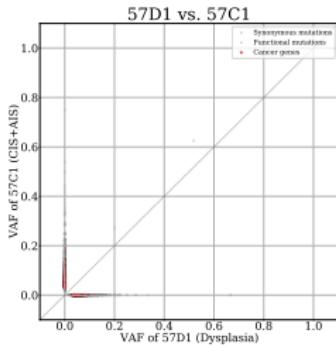
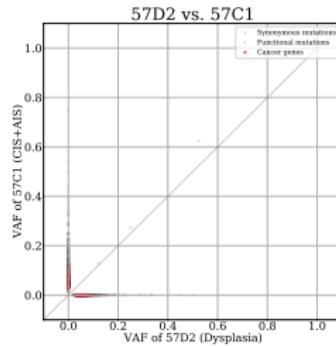


Figure: VAF distribution of validated mutations (Ding et al., 2012)

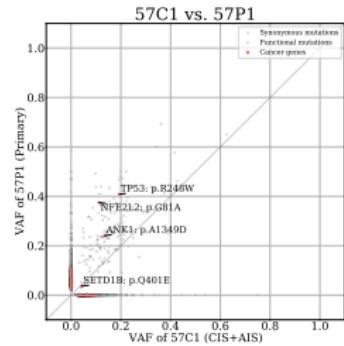
VAF Plots I



(a) Dysplasia + CIS



(b) Dysplasia + CIS



(c) CIS + Primary

Figure: VAF plots in patient #57

PyClone?

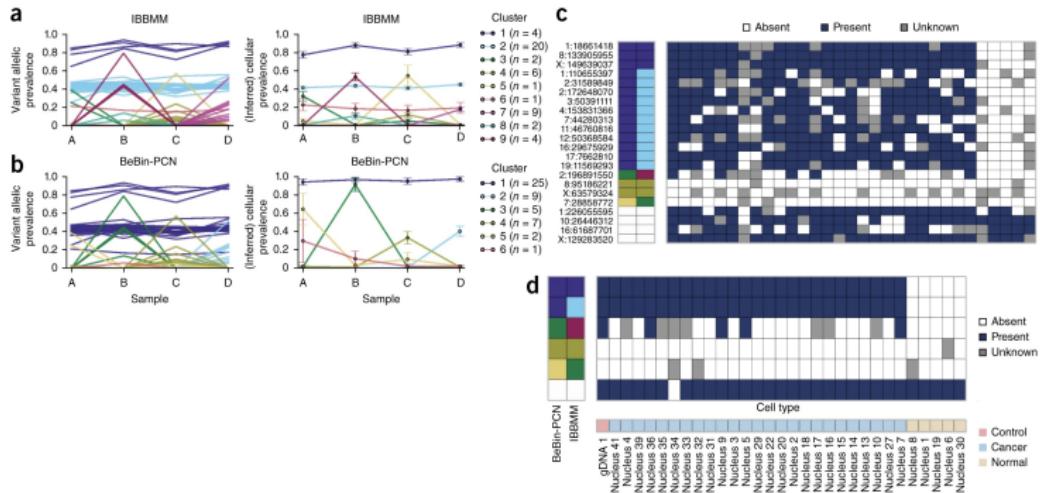


Figure: Analysis of multiple samples by PyClone (Roth et al., 2014)

PyClone Plots I

Findings in VAF Analysis

4. Results

4.6. Tumor Evolution Trajectories Analysis

Revolver?

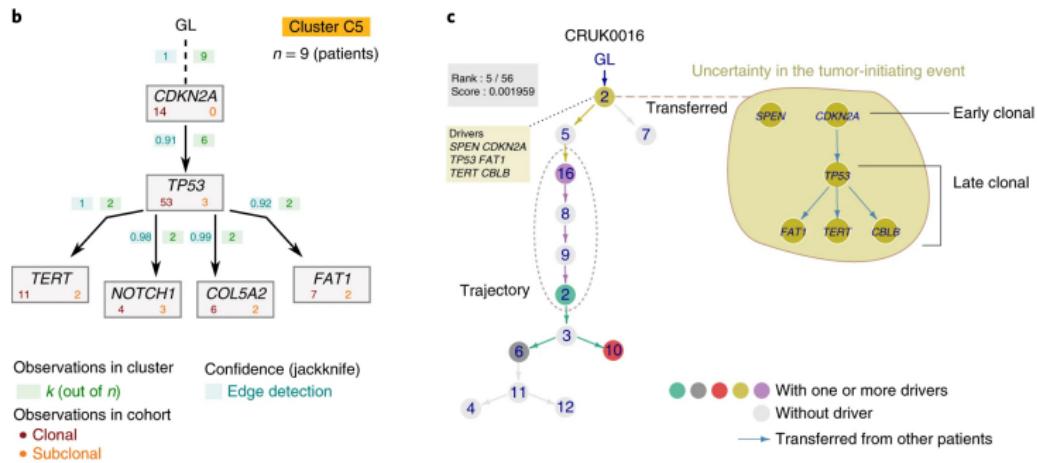


Figure: Repeated Evolutionary Trajectories (Caravagna et al., 2018)

Revolver in LUSC I

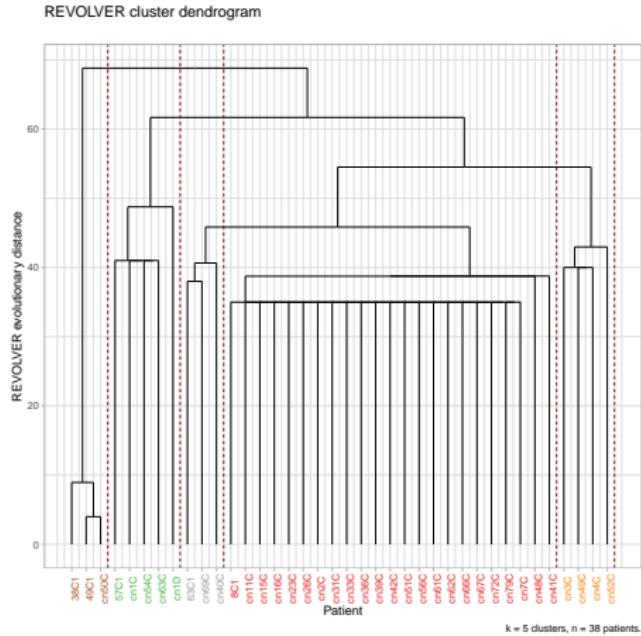


Figure: Dendrogram analysis in LUSC

Revolver in LUSC II

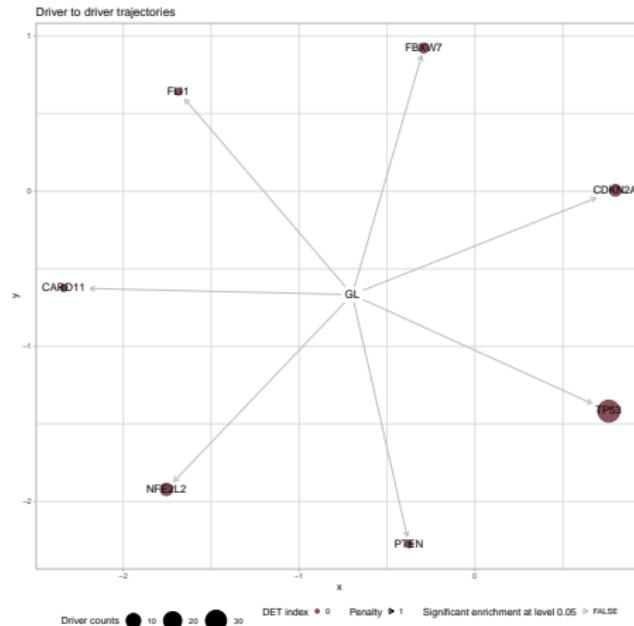


Figure: Driver analysis in LUSC

Revolver in LUAD I

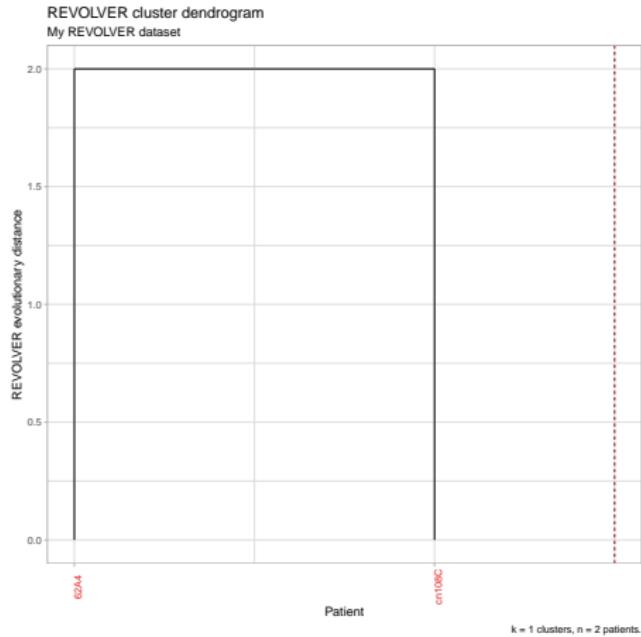


Figure: Dendrogram analysis in LUAD

Revolver in LUAD II

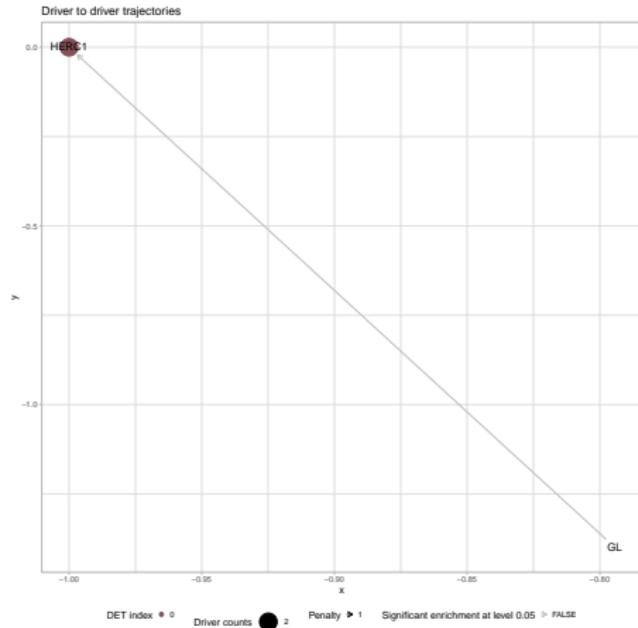


Figure: Driver analysis in LUAD

Findings in Tumor Evolution Trajectories Analysis

4. Results

4.7. Differences in Gene Expression Levels

RSEM?

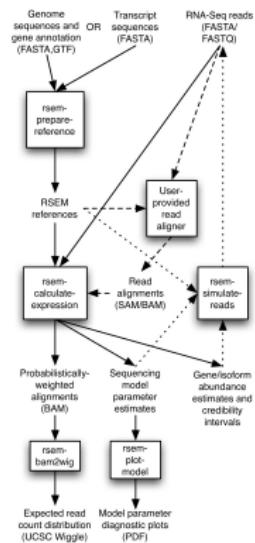


Figure: RSEM workflow (Li & Dewey, 2011)

DESeq2?

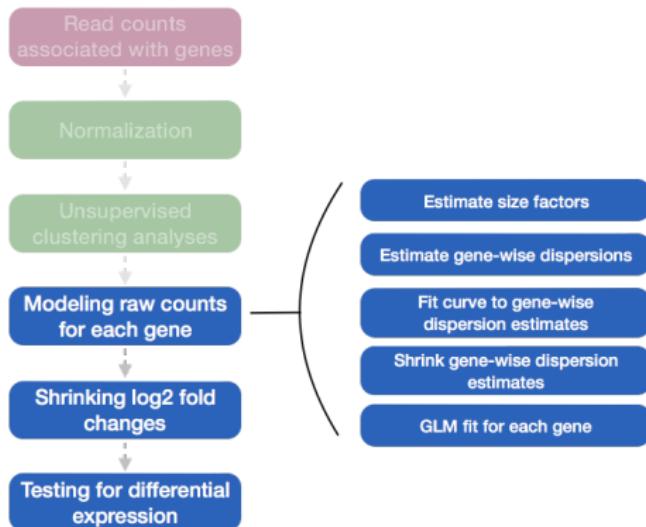


Figure: DESeq2 workflow (Love, Huber, & Anders, 2014)

DEG Selection Strategy

DEG: differentially expressed genes

Fold Change

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

P-value

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

Adjusted P-value

$$P_{adj} < 0.05$$

Enrichr?

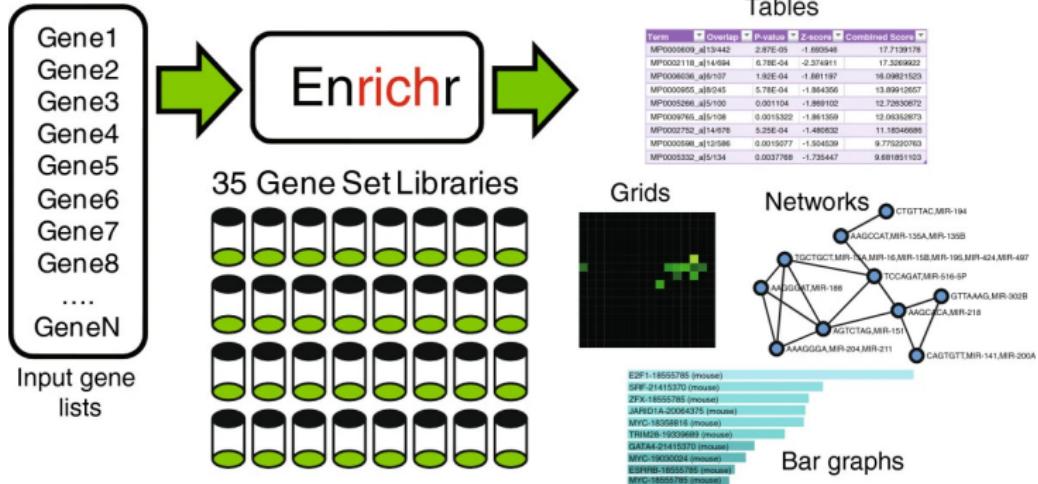


Figure: Enrichr workflow (E. Y. Chen et al., 2013; Kuleshov et al., 2016)

Gene-set Library

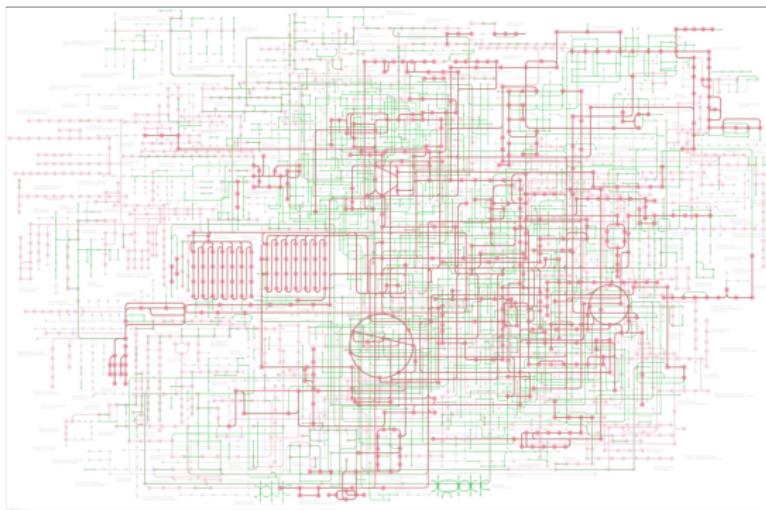


Figure: The global map of metabolic pathways by KEGG (Kanehisa et al., 2021)

KEGG

KEGG 2021 Human

WTS Data Composition

Table: Number of WTS samples

| Cancer Subtype | Stage | Number of Samples | |
|----------------|-----------|-------------------|----|
| | | Normal | 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? | 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 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

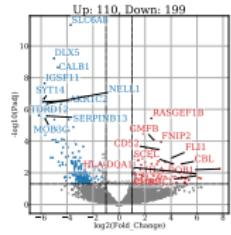
| gene | log2FoldChange | pvalue | padj |
|---------|----------------|----------|----------|
| AKR1C1 | 6.18e+00 | 5.14e-26 | 5.01e-23 |
| AKR1C2 | 6.06e+00 | 1.19e-22 | 5.04e-20 |
| CYP4F11 | 5.58e+00 | 1.51e-20 | 4.36e-18 |

Table: Down-regulated DEG in LUSC

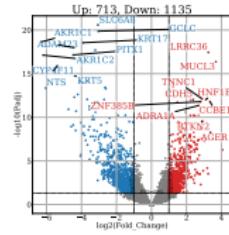
| gene | log2FoldChange | pvalue | padj |
|---------|----------------|----------|----------|
| SFTPC | -5.85e+00 | 9.16e-21 | 2.83e-18 |
| FAM107A | -4.62e+00 | 2.27e-33 | 9.60e-30 |
| LRRC36 | -4.53e+00 | 5.49e-36 | 3.48e-32 |

DEG Volcano Plots in LUSC

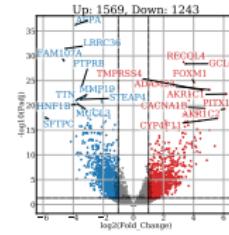
Normal → Dysplasia → CIS → Primary (LUSC)



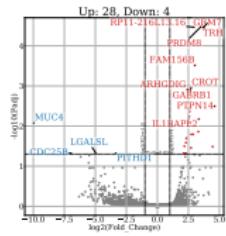
(a) Normal-Dysplasia



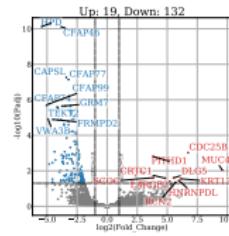
(b) Normal-CIS



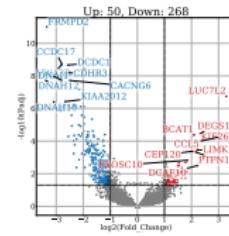
(c) Normal-Primary



(d) Dysplasia-CIS



(e) Dysplasia-Primary

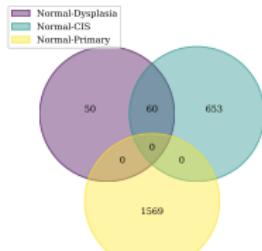


(f) CIS-Primary

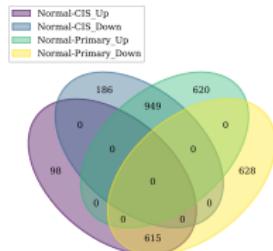
Figure: DEG Volcano Plots in LUSC

DEG Venn Diagram in LUSC

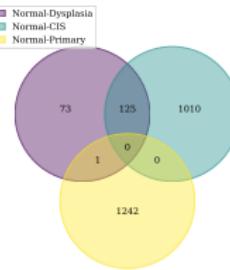
Normal → Dysplasia → CIS → Primary (LUSC)



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

SFTPC

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

4. Results

4.7. Differences in Gene Expression Levels

4.7.2. Comparing cancer stage in LUAD

DEG List in LUSC

Table: Up-regulated DEG in LUAD

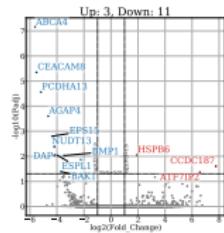
| gene | log2FoldChange | pvalue | padj |
|-------|----------------|----------|----------|
| ABCA4 | 4.95e+00 | 3.01e-12 | 2.58e-09 |
| HMGA2 | 4.79e+00 | 8.06e-08 | 1.46e-05 |
| KIF12 | 4.48e+00 | 1.33e-06 | 1.46e-04 |

Table: Down-regulated DEG in LUAD

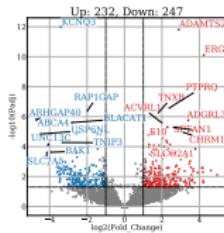
| gene | log2FoldChange | pvalue | padj |
|--------|----------------|----------|----------|
| SLC6A4 | -6.20e+00 | 5.80e-10 | 2.36e-07 |
| IL1RL1 | -4.20e+00 | 7.47e-06 | 5.82e-04 |
| RNF185 | -4.06e+00 | 4.75e-05 | 2.45e-03 |

DEG Volcano Plots in LUAD

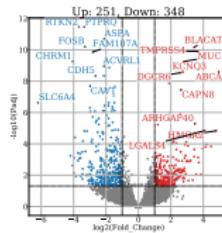
Normal → AAH → AIS → Primary (LUAD)



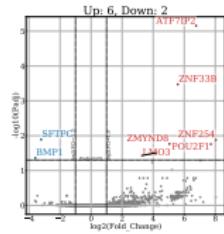
(a) Normal-AAH



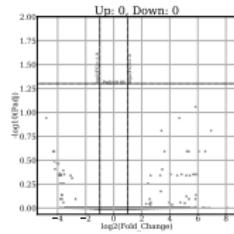
(b) Normal-AIS



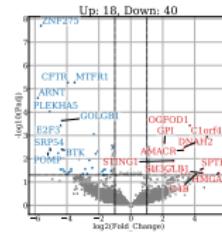
(c) Normal-Primary



(d) AAH-AIS



(e) AAH-Primary

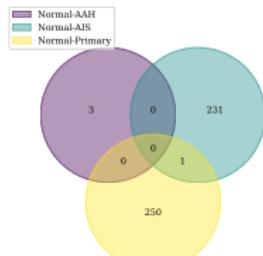


(f) AIS-Primary

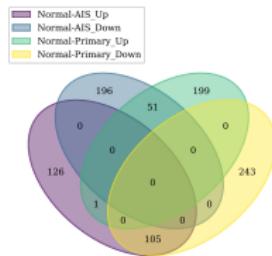
Figure: DEG Volcano Plots in LUAD

DEG Venn Diagram in LUAD

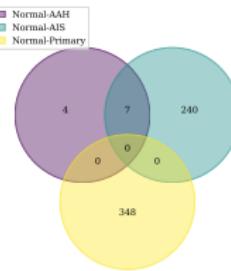
Normal → AAH → AIS → Primary (LUAD)



(a) Up-regulated



(b) Both



(c) Down-regulated

Figure: DEG Venn Diagram in LUAD

Enrichment test with Normal vs. AAH in LUAD

Table: Up-regulated Pathways on Normal vs. AAH

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

Table: Down-regulated Pathways on Normal vs. AAH

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

Enrichment test with Normal vs. AIS in LUAD

Table: Up-regulated Pathways on Normal vs. AIS

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

Table: Down-regulated Pathways on Normal vs. AIS

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

Enrichment test with Normal vs. Primary in LUAD

Table: Up-regulated Pathways on Normal vs. Primary

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

Table: Down-regulated Pathways on Normal vs. Primary

| Term name | 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 |

Finding in Comparing cancer stage in LUAD I

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).

KCNQ3

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

CHRM1

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

4. Results

4.7. Differences in Gene Expression Levels

4.7.3. Recur vs. Non-recur in LUSC

Table: Number of WTS LUSC samples

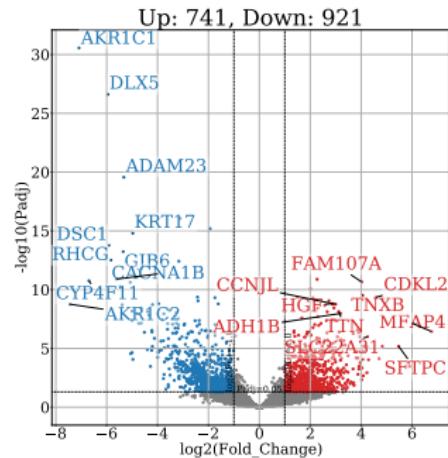
| Recurrence? | Stage | Number of Samples | |
|----------------|-----------|-------------------|-----------|
| | | Normal | Dysplasia |
| Recurrence | Normal | 1 | |
| | Dysplasia | | 1 |
| | CIS+AIS | 5 | |
| | Primary | 6 | |
| | Total | 13 | |
| Non-recurrence | Normal | 16 | |
| | Dysplasia | | 1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

Pooled normal samples

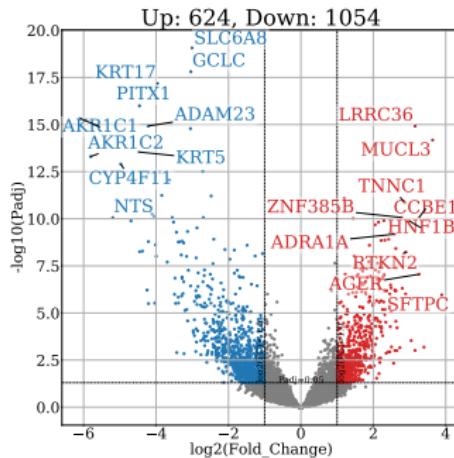
In order to compare with Normal stage, merging Normal samples.

∴ Insufficient number of Normal samples in Recur.

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



(a) Recur



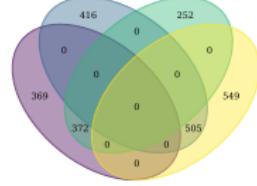
(b) Non-recur

Figure: DEG Volcanot Plot with CIS in LUSC

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



(a) Up-regulated



(b) Both



(c) Down-regulated

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

Enrichment test for Recur-specific with CIS in LUSC

Table: Up-regulated Pathways for Recur-specific

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

Table: Down-regulated Pathways for Recur-specific

| Term name | 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

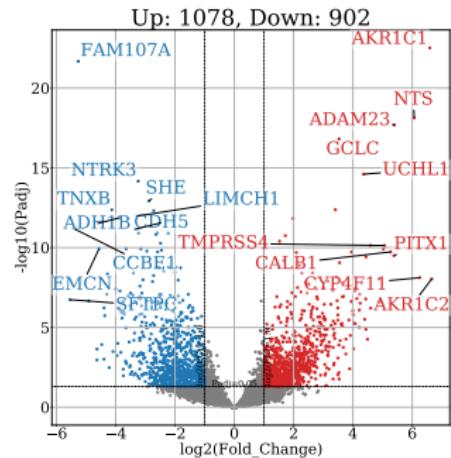
Table: Up-regulated Pathways for Intersected

| 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 |

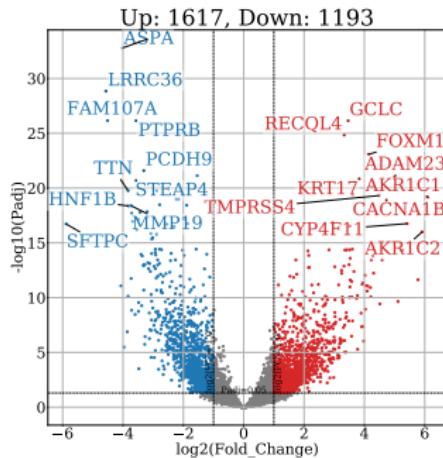
Table: Down-regulated Pathways for Intersected

| 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



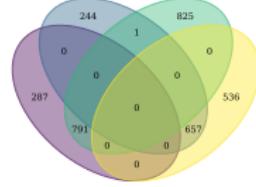
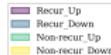
(b) Non-recur

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

4. Results

4.7. Differences in Gene Expression Levels

4.7.4. Within Recur in LUSC

DEG List for CIS within Recur in LUSC

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

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

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

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

DEG List for Primary within Recur in LUSC

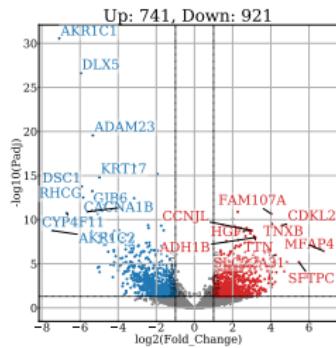
Table: Up-regulated DEG for Primary within Recur in LUSC

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

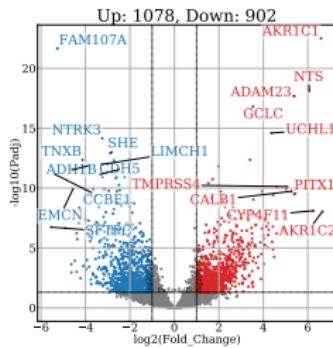
Table: Down-regulated DEG for Primary within Recur in LUSC

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

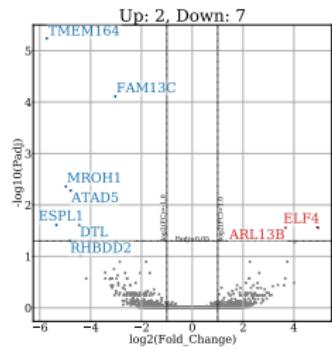
DEG Volcano Plots with Recur in LUSC



(a) Normal-CIS



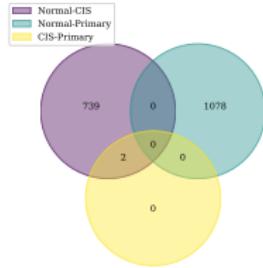
(b) Normal-Primary



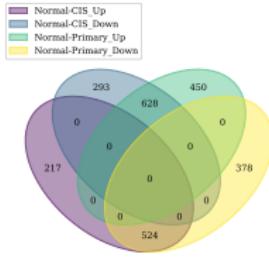
(c) CIS-Primary

Figure: DEG Volcano Plots with Recur samples in LUSC

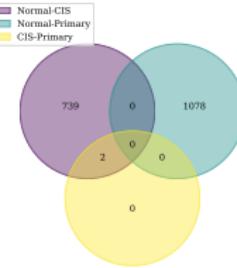
DEG Venn Diagram with Recur in LUSC



(a) Up-regulated



(b) Both



(c) Down-regulated

Figure: DEG Venn Diagram with Recur samples in LUSC

Enrichment test with Normal vs. CIS for Recur

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

| Term name | 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.
- ② Regulate steroids (Jin et al., 2009) and hormones (Penning et al., 2000).
- ③ Promote the metastasis of NSCLC (Z. Hong et al., 2018)

ADAM23

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

FAM107A

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

4. Results

4.7. Differences in Gene Expression Levels

4.7.5. Within Non-recur in LUSC

DEG List for CIS within Non-recr in LUSC

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

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

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

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

DEG List for Primary within Non-recur in LUSC

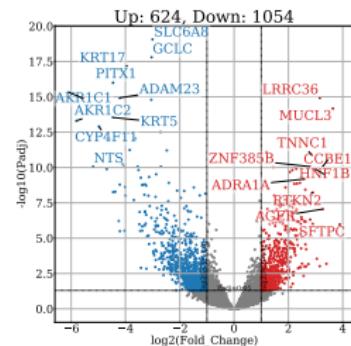
Table: Up-regulated DEG for Primary within Non-recur in LUSC

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

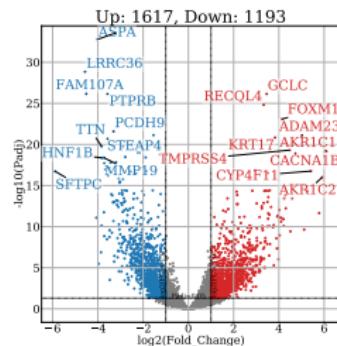
Table: Down-regulated DEG for Primary within Non-recur in LUSC

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

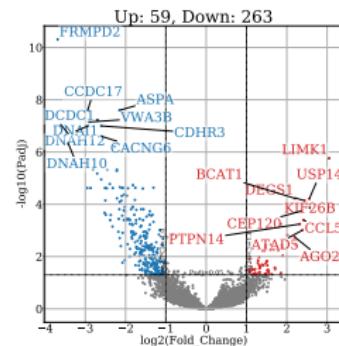
DEG Volcano Plots with Non-recur in LUSC



(a) Normal-CIS



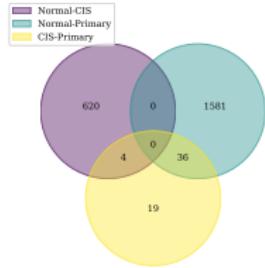
(b) Normal-Primary



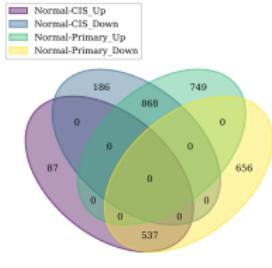
(c) CIS-Primary

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

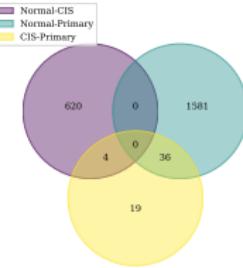
DEG Venn Diagram with Non-recur in LUSC



(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-recur in LUSC I

AKR1C1 & AKR1C2

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

CYP4F11

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

LRRC36

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

4. Results

4.7. Differences in Gene Expression Levels

4.7.6. Within Non-recur in LUAD

LUAD Data Composition

Table: Number of WTS LUAD samples

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

DEG List for AIS within Non-recur in LUAD

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

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

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

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

DEG List for Primary within Non-recur in LUAD

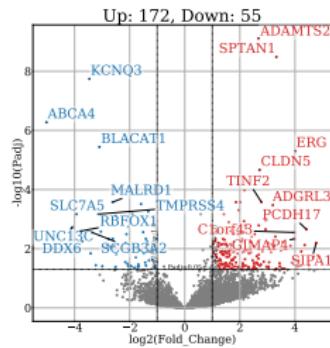
Table: Up-regulated DEG for Primary within Non-recur in LUAD

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

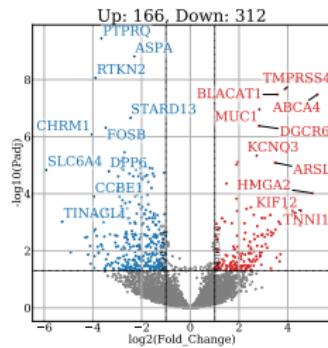
Table: Down-regulated DEG for Primary within Non-recur in LUAD

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

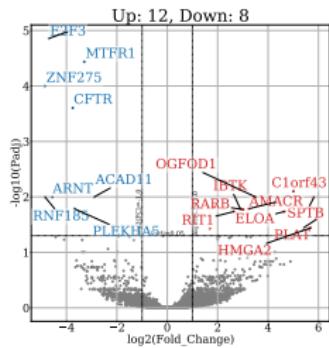
DEG Volcano Plots with Non-recr in LUAD



(a) Normal-AIS



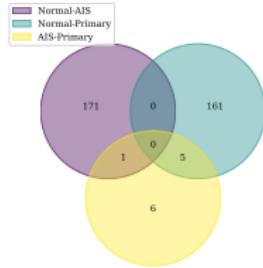
(b) Normal-Primary



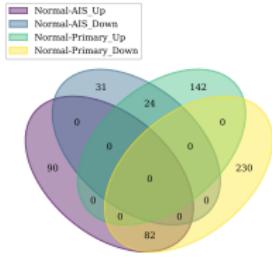
(c) AIS-Primary

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

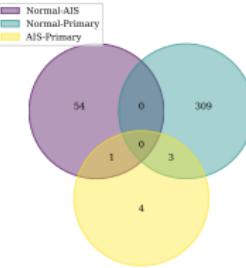
DEG Venn Diagram with Non-recur in LUAD



(a) Up-regulated



(b) Both



(c) Down-regulated

Figure: DEG Venn Diagram with Non-recur in LUAD

Enrichment test with Normal vs. AIS in LUAD

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

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

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

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

Enrichment test with Normal vs. Primary in LUAD

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

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

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

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

KCNQ3

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

BLACAT1

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

Findings in DEG Analysis

4. Results

4.8. Bulk Cell Deconvolution

BisqueRNA?

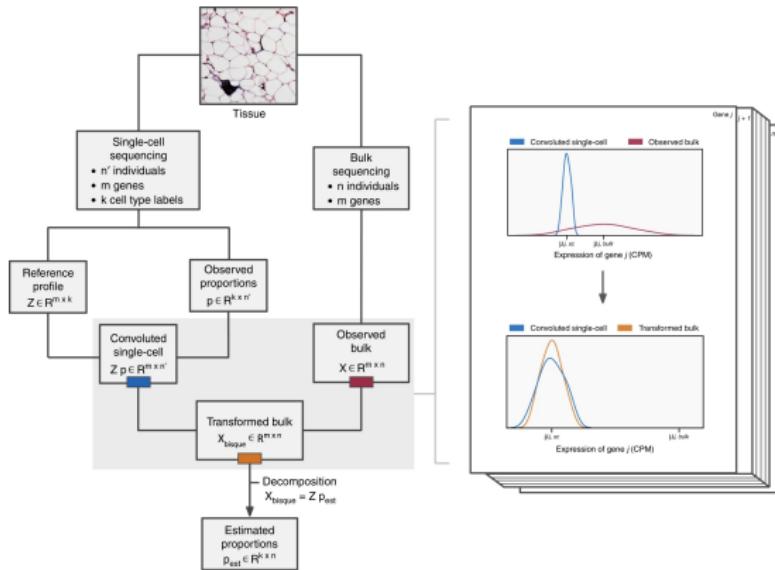


Figure: Workflow for BisqueRNA (Jew et al., 2020)

4. Results

4.8. Bulk Cell Deconvolution

4.8.1. Reference by Kim et al. (2020)

Reference Single-cell Data

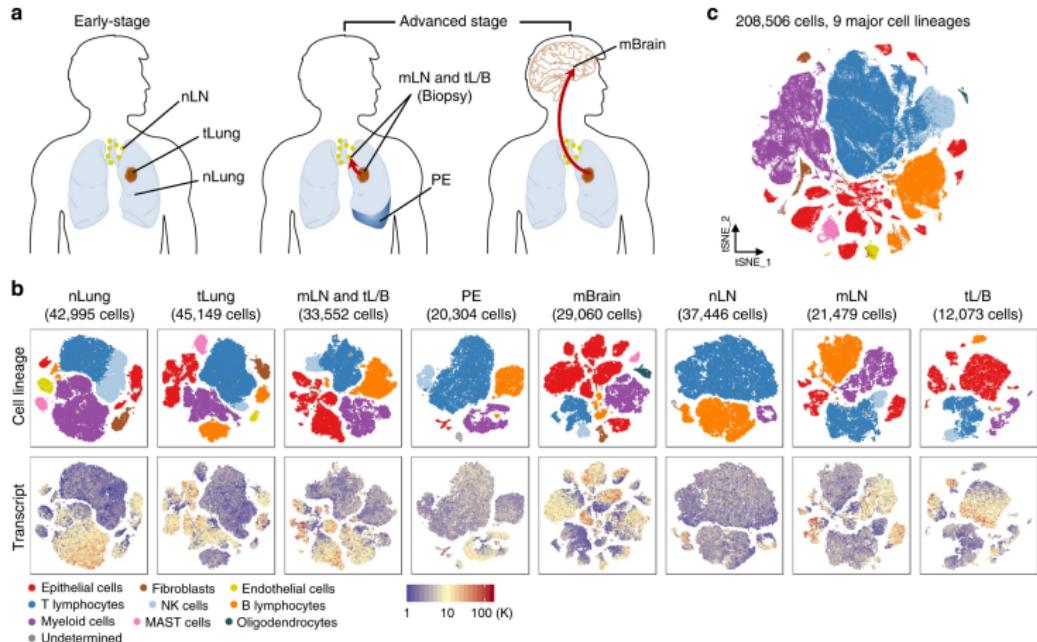


Figure: Comprehensive dissection and clustering of 208,506 single cells from LUAD patients (Kim et al., 2020)

Cluster Plot in LUSC

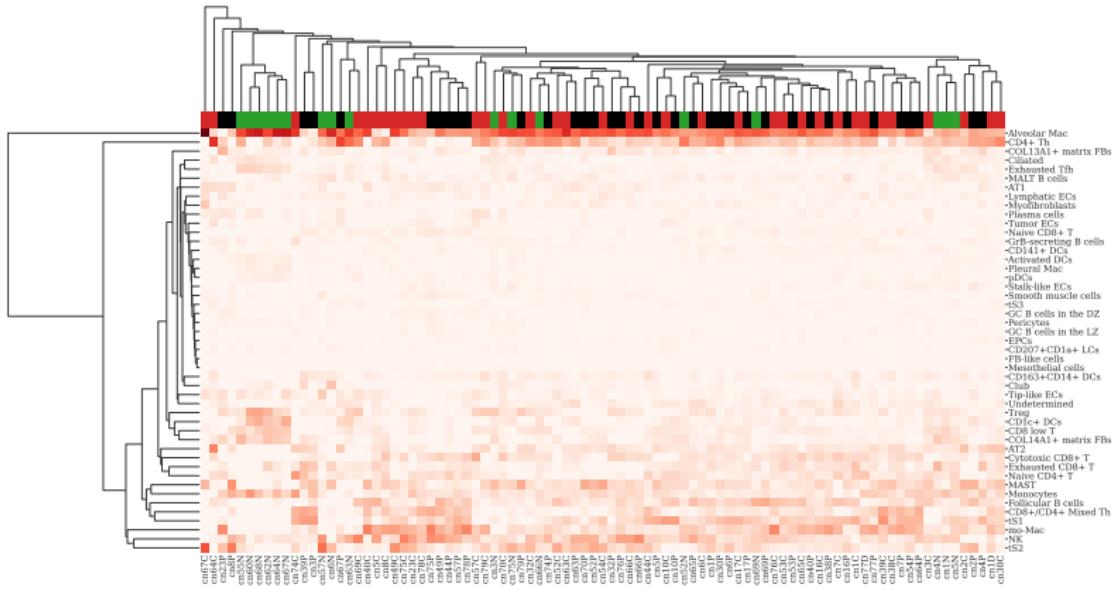
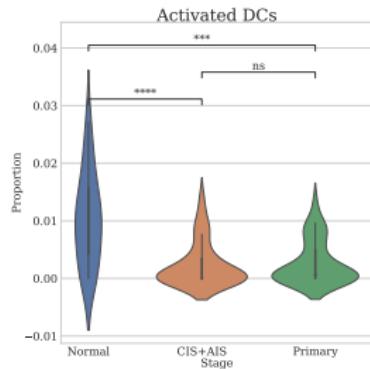
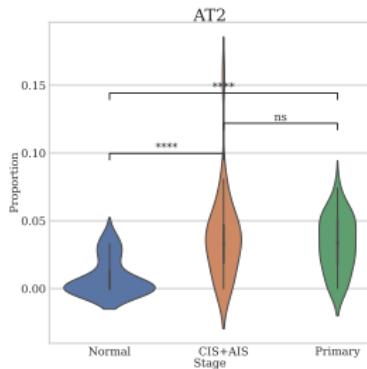


Figure: Cluster Plot in LUSC

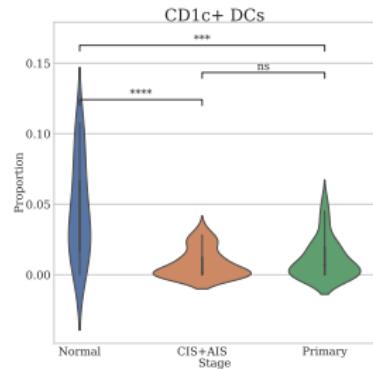
Violin Plots in LUSC I



(a) Activated DCs



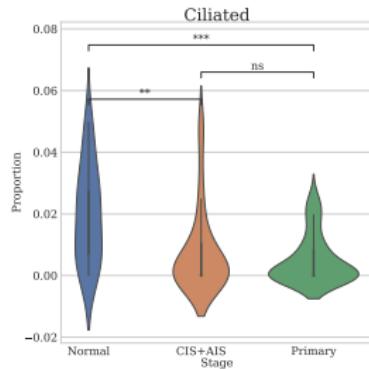
(b) Alveolar type II



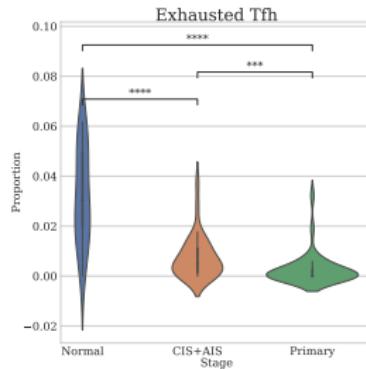
(c) Langerhans cells

Figure: Violin Plots in LUSC

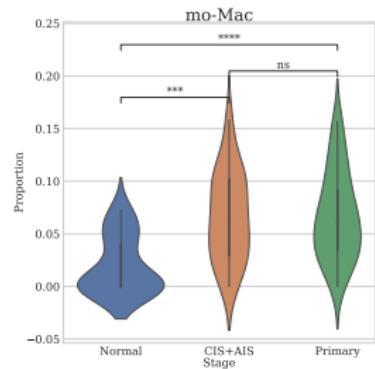
Violin Plots in LUSC II



(d) Ciliated cells



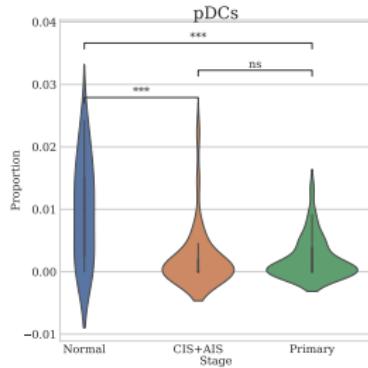
(e) Exhausted T follicular helper



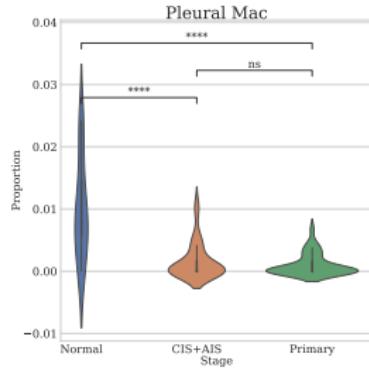
(f) Mo & Mac

Figure: Violin Plots in LUSC

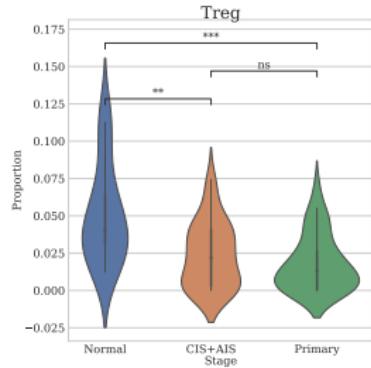
Violin Plots in LUSC III



(g) Plasmacytoid DCs



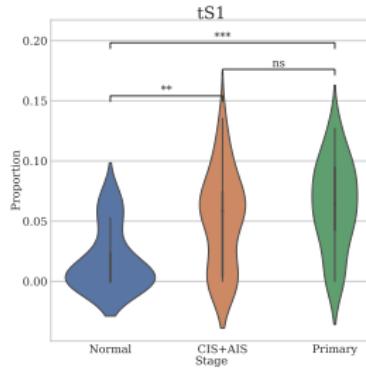
(h) Pleural Mac



(i) Regulatory T cells

Figure: Violin Plots in LUSC

Violin Plots in LUSC IV



(j) Transcriptional states 1

Figure: Violin Plots in LUSC

Findings in Bulk Cell Deconvolution with LUSC I

Activated DCs

- ① Activated DCs have higher proportion in Normal samples.

Alveolar type II

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

CD1c+ DCs (Langerhans cells; LCs)

- ① LCs have higher proportion in Normal samples.

Ciliated cells

- ① Ciliated cells have higher proportion in Normal samples.

Findings in Bulk Cell Deconvolution with LUSC II

Exhausted T follicular help cells

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

Monocyte & Macrophage

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

Plasmacytoid DCs

- ① Plasmacytoid DCs have higher proportion in Normal samples.

Pleural Macrophages

- ① Pleural Macrophages have higher proportion in Normal samples.

Findings in Bulk Cell Deconvolution with LUSC III

Regulatory T cells

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

Transcriptional states 1 (tS1)

- ① tS1 have lower proportion in Normal samples.

Cluster Plot in LUAD

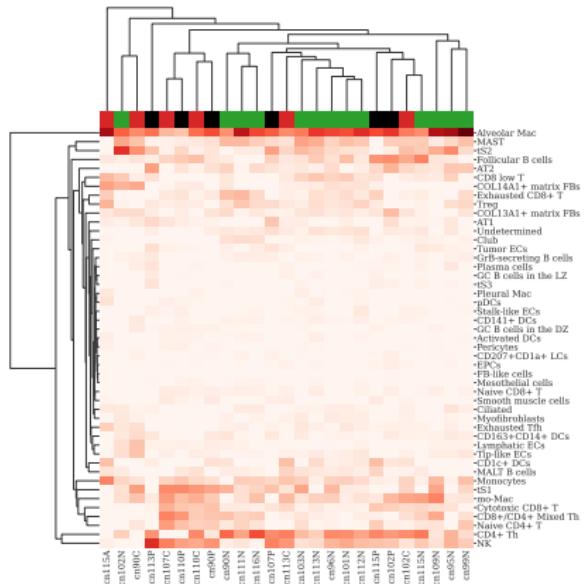
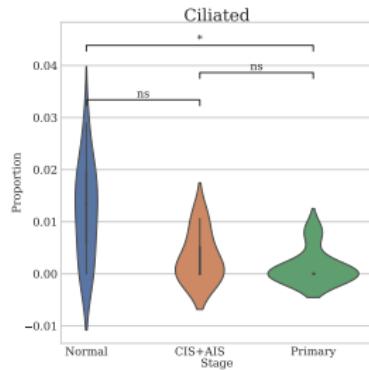
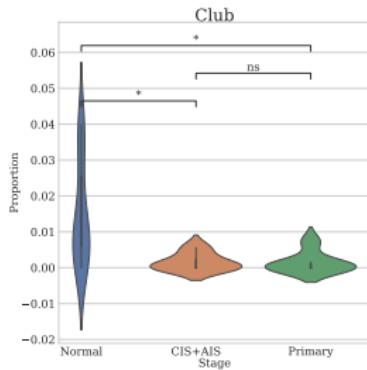


Figure: Cluster Plot in LUAD

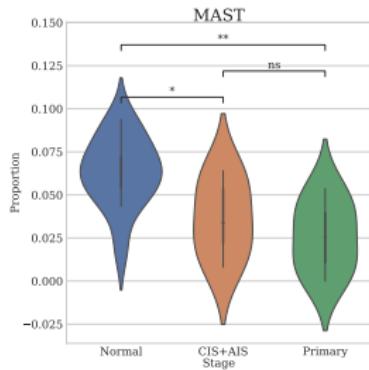
Violin Plots in LUAD I



(a) Ciliated cells



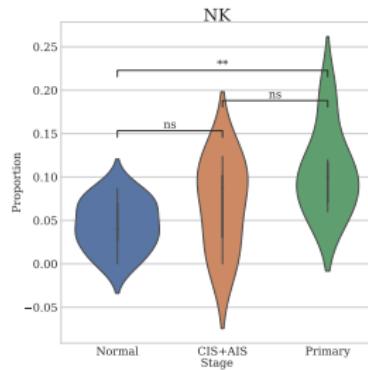
(b) Club Cell



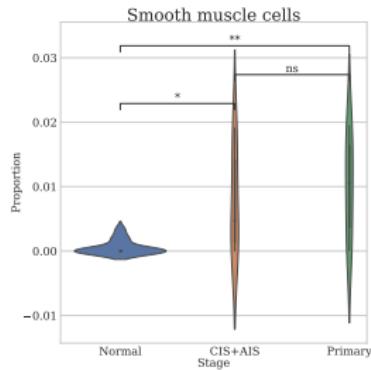
(c) Mast cell

Figure: Violin Plots in LUAD

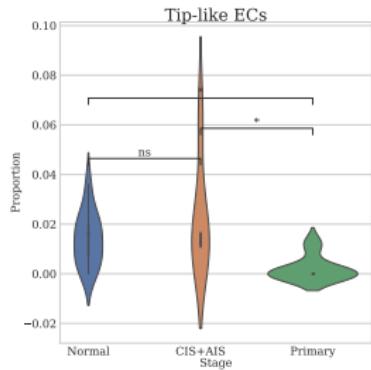
Violin Plots in LUAD II



(d) NK cells



(e) Smooth muscle cells



(f) Tip-like ECs

Figure: Violin Plots in LUAD

Findings in Bulk Cell Deconvolution with LUAD I

Ciliated cells

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

Club cells

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

Mast cells

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

Natural Killer cells

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

Smooth muscle cells

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

Tip-like ECs

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

4. Results

4.8. Bulk Cell Deconvolution

4.8.2. Reference by Gueguen et al. (2021)

Reference Single-cell Data

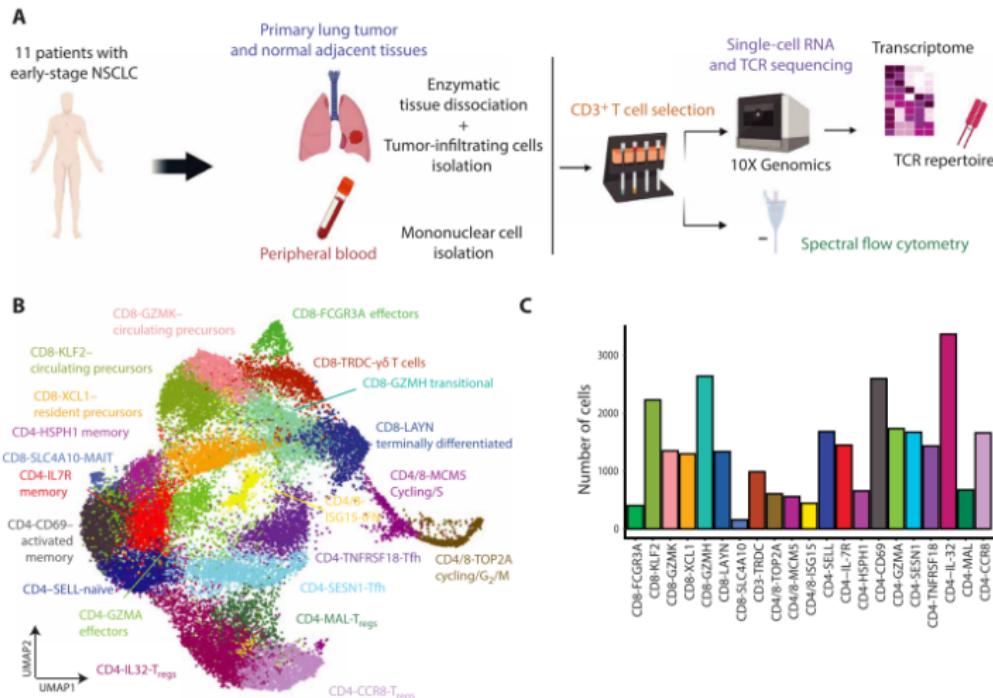


Figure: Characterization of CD3⁺ TILs in NSCLC (Gueguen et al., 2021)

Cluster Plots in LUSC

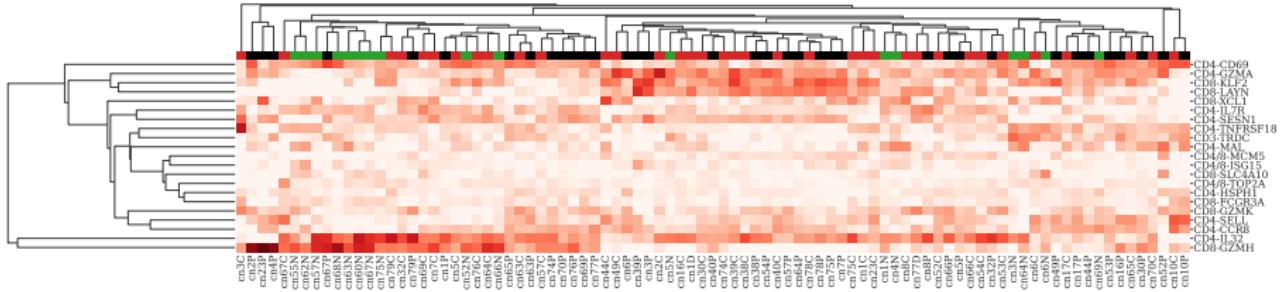
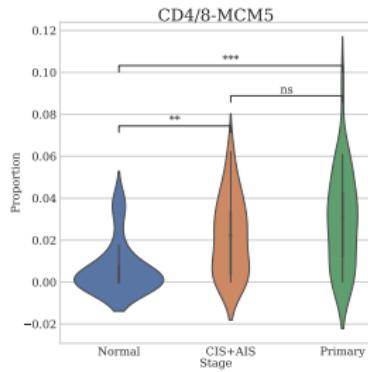
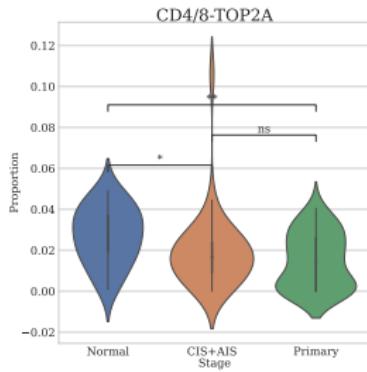


Figure: Cluster Plot in LUAD

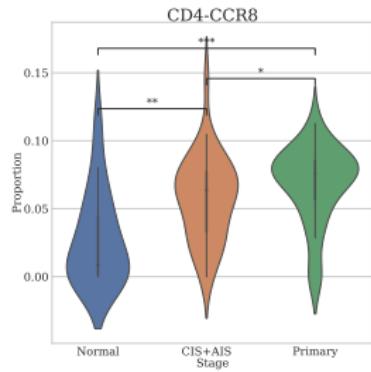
Violin Plots in LUSC I



(a) CD4/8-MCM5



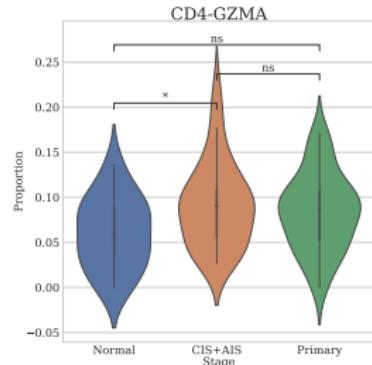
(b) CD4/8-TOP2A



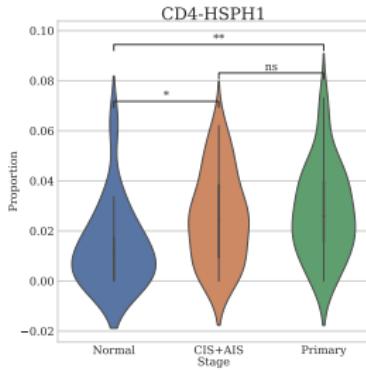
(c) CD4-CCR8

Figure: Violin Plots in LUSC

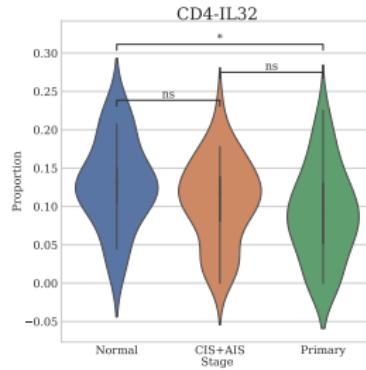
Violin Plots in LUSC II



(d) CD4-GZMA



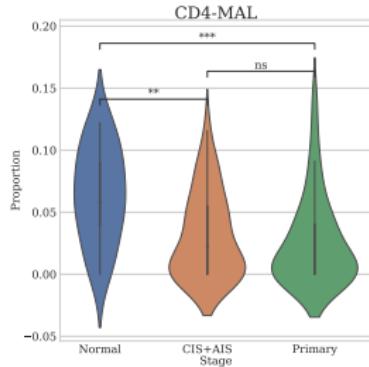
(e) CD4-HSPH1



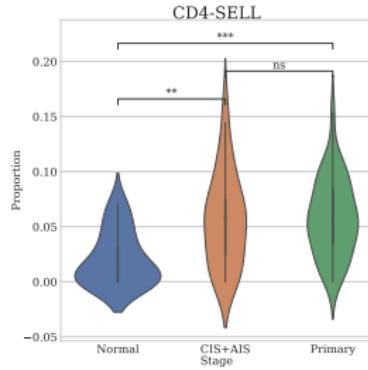
(f) CD4-IL32

Figure: Violin Plots in LUSC

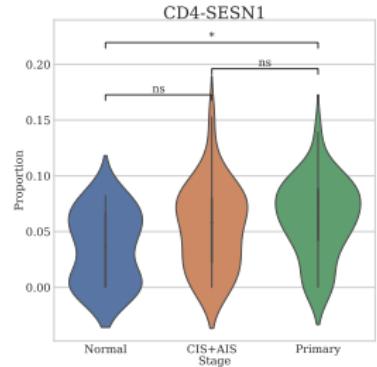
Violin Plots in LUSC III



(g) CD4-MAL



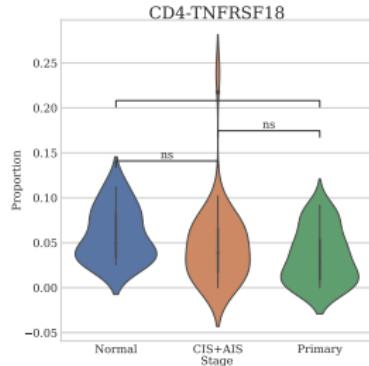
(h) CD4-SELL



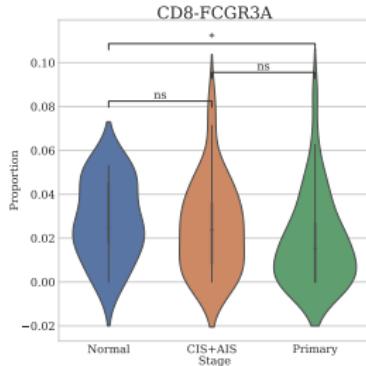
(i) CD4-SESN1

Figure: Violin Plots in LUSC

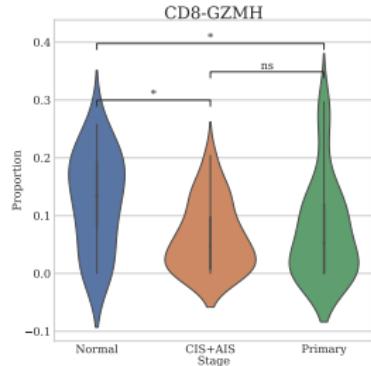
Violin Plots in LUSC IV



(g) CD4-TNFRSF18



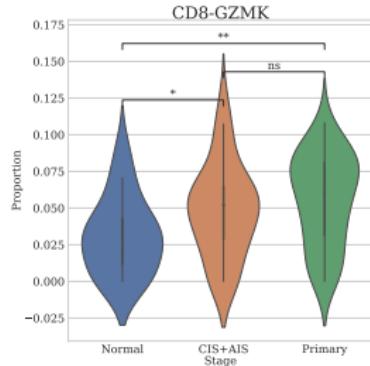
(h) CD8-FCGR3A



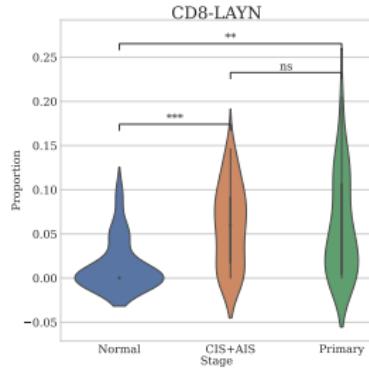
(i) CD8-GZMH

Figure: Violin Plots in LUSC

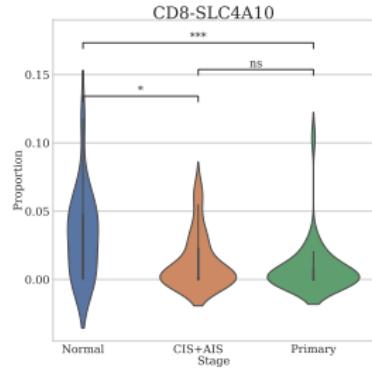
Violin Plots in LUSC V



(g) CD8-GZMK



(h) CD8-LAYN



(i) CD8-SLC4A10

Figure: Violin Plots in LUSC

Findings in Bulk Cell Deconvolution with LUSC I

CD4/8-MCM5

content...

CD4/8-TOP2A

content...

CD4-CCR8

content...

CD4-GZMA

content...

CD4-HSPH1

content...

Findings in Bulk Cell Deconvolution with LUSC II

CD4-IL32

content...

CD4-MAL

content...

CD4-SELL

content...

CD4-SESN1

content...

CD4-TNFRSF18

content...

Findings in Bulk Cell Deconvolution with LUSC III

CD8-FCGR3A

content...

CD8-GZMH

content...

CD8-GZMK

content...

CD8-LAYN

content...

CD8-SLC4A10

content...

Cluster Plots in LUAD

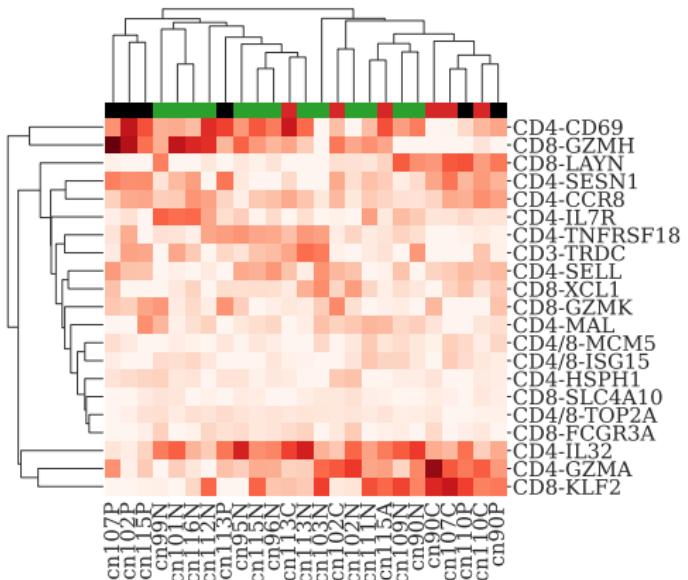
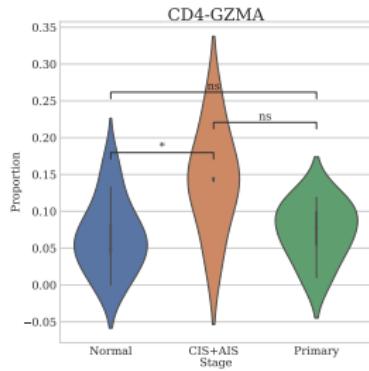
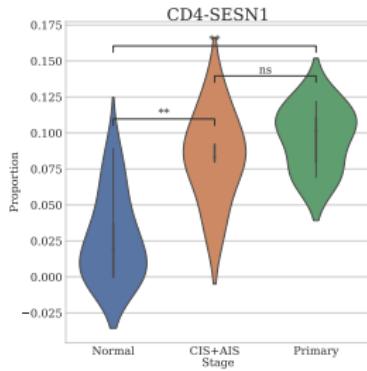


Figure: Cluster Plot in LUAD

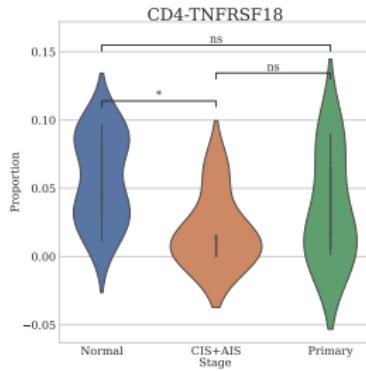
Violin Plots in LUAD



(a) CD4-GZMA



(b) CD4-SESN1



(c) CD4-TNFRSF18

Figure: Violin Plots in LUAD

Findings in Bulk Cell Deconvolution with LUAD I

CD4-GZM

content...

CD4-SESN1

content...

CD4-TNFRSF18

content...

Findings in Bulk Cell Deconvolution

4. Results

4.9. Discovery of Gene Fusion

Arriba?

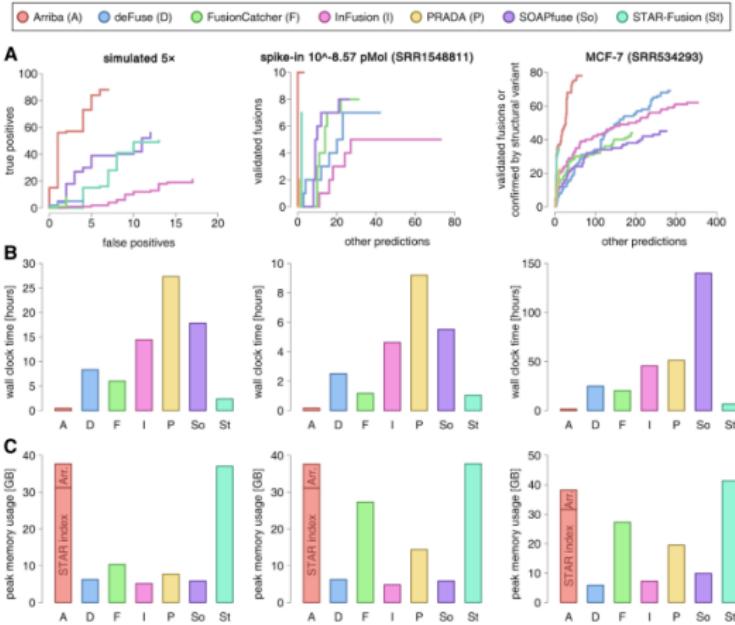


Figure: Benchmark of Arriba versus alternative methods (Uhrig et al., 2021)

Findings in Gene Fusion Discovery

4. Results

4.10. Discovery of Mutational Signature

Mutation Signature?

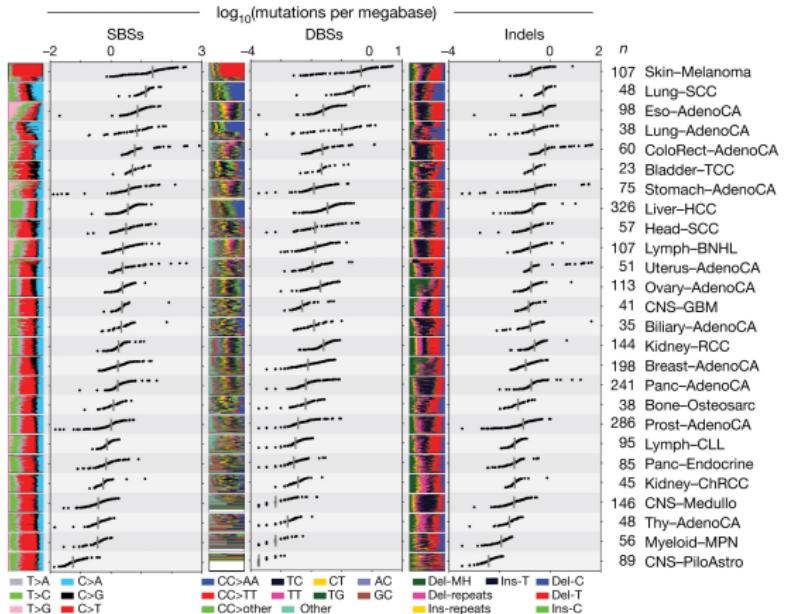


Figure: Mutation Burdens across PCAWG tumor types (Alexandrov et al., 2020)

SigProfiler?

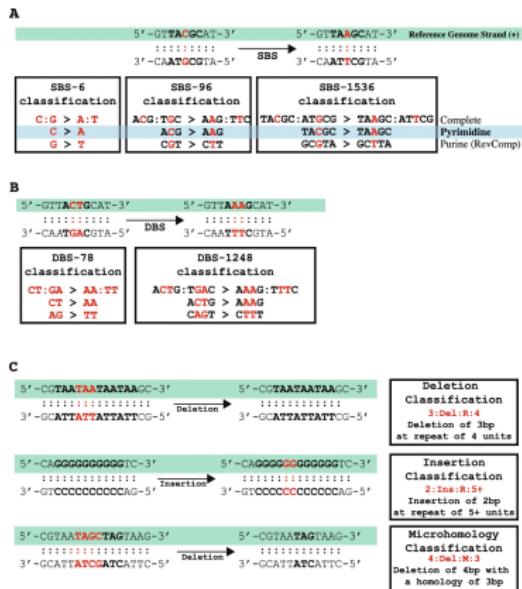


Figure: Classification of mutation signatures by SigProfiler (Bergstrom et al., 2019; Islam et al., 2021; Bergstrom et al., 2020)

4. Results

4.10. Discovery of Mutational Signature

4.10.1. Single Base Substitutions (SBS)

SBS Signatures I

SBS1

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

SBS2

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

SBS Signatures II

SBS4

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

SBS5

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

SBS10b

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

SBS Signatures III

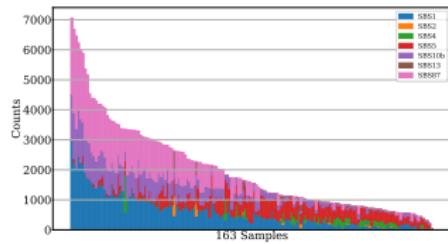
SBS13

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

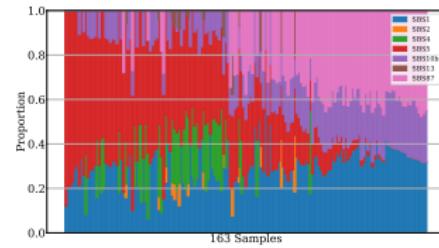
SBS87

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

SBS in LUSC I



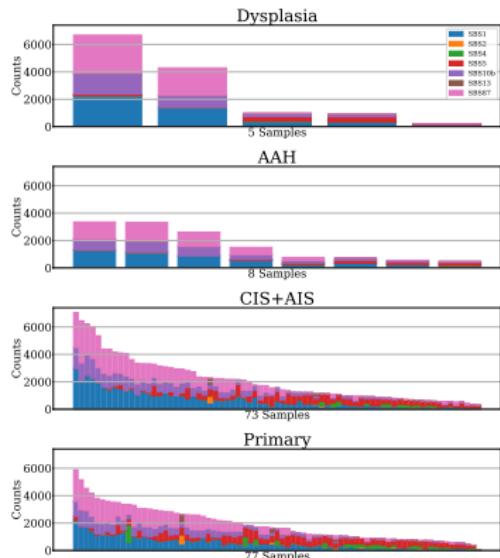
(a) Absolute



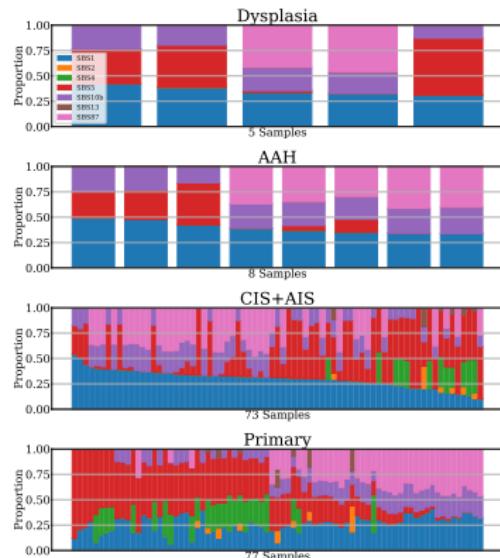
(b) Relative

Figure: SBS Bar Plot in LUSC

SBS in LUSC II



(a) Absolute



(b) Relative

Figure: SBS Bar Plot by Cancer Subtype in LUSC

SBS in LUSC with Smoking I

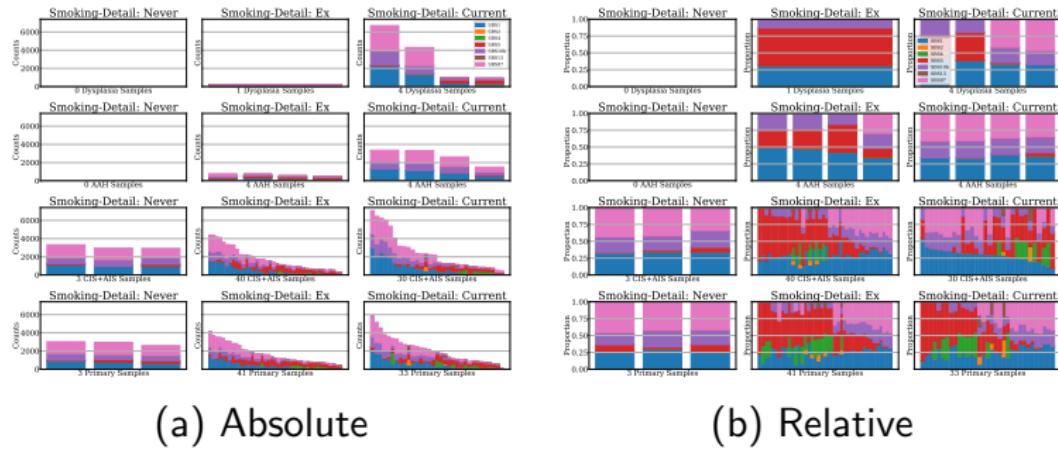
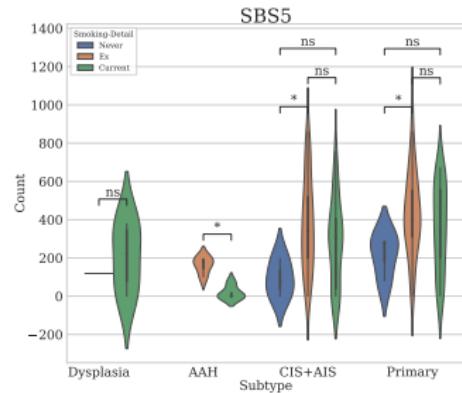
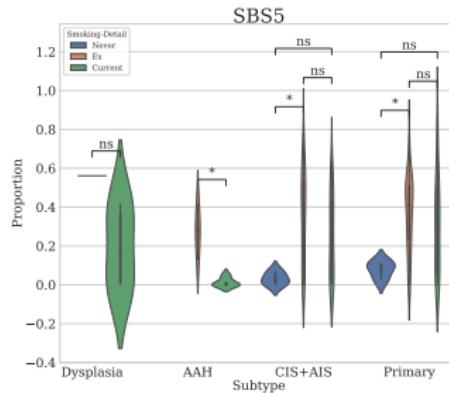


Figure: SBS Bar Plot by Cancer Subtype & Smoking in LUSC

SBS in LUSC with Smoking II



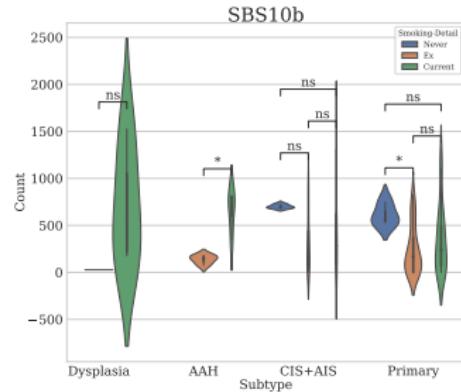
(a) Absolute



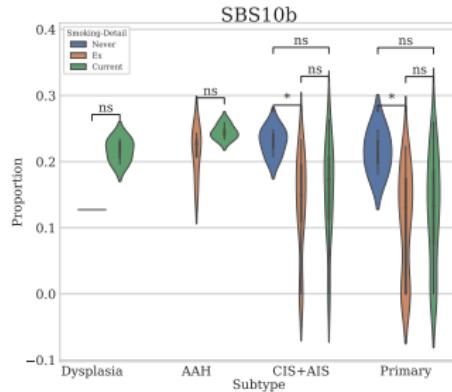
(b) Relative

Figure: SBS5 Signature in LUSC with Smoking

SBS in LUSC with Smoking III



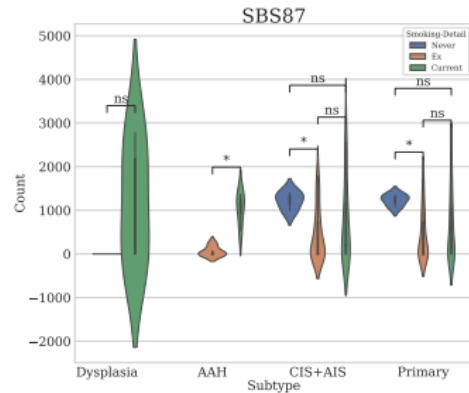
(a) Absolute



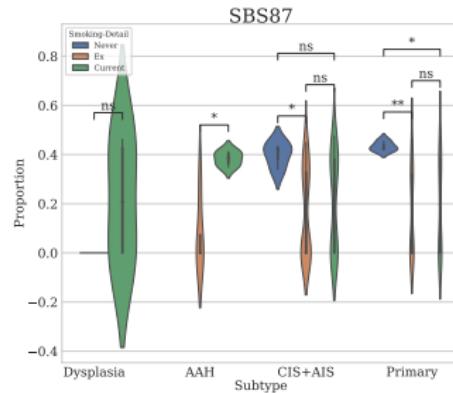
(b) Relative

Figure: SBS10b Signature in LUSC with Smoking

SBS in LUSC with Smoking IV



(a) Absolute



(b) Relative

Figure: SBS87 Signature in LUSC with Smoking

SBS in LUSC with Recurrence I

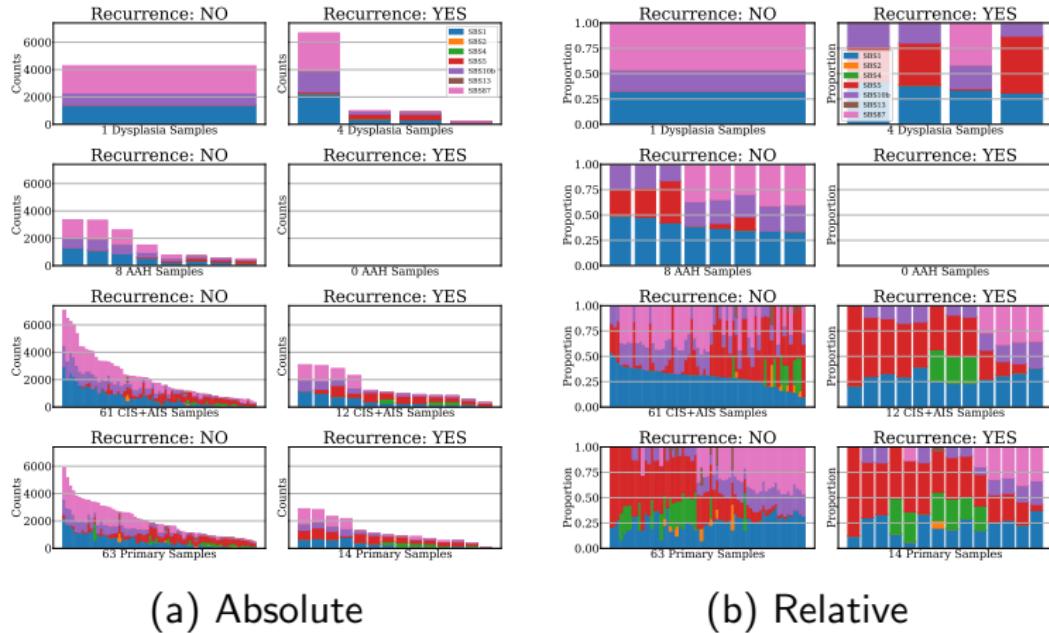
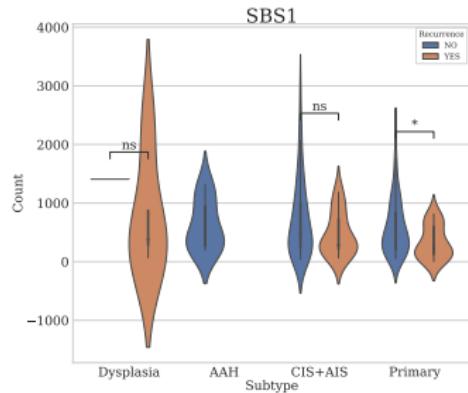
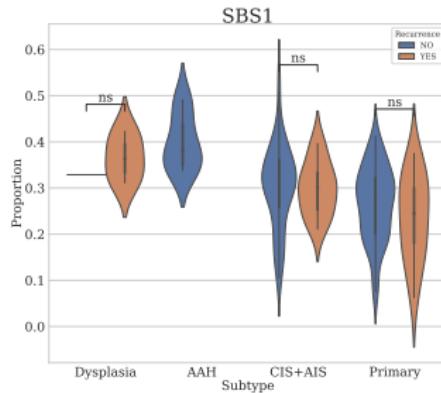


Figure: SBS Bar Plot by Cancer Subtype & Recurrence in LUSC

SBS in LUSC with Recurrence II



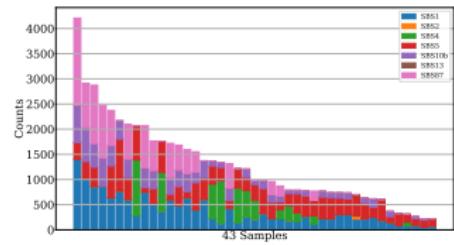
(a) Absolute



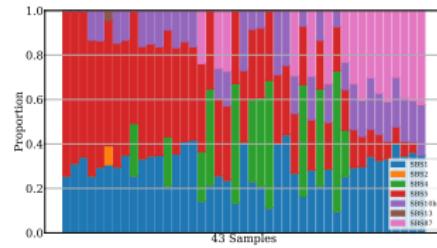
(b) Relative

Figure: SBS1 Signature in LUSC with Recurrence

SBS in LUAD I



(a) Absolute



(b) Relative

Figure: SBS Bar Plot in LUSC

SBS in LUAD II

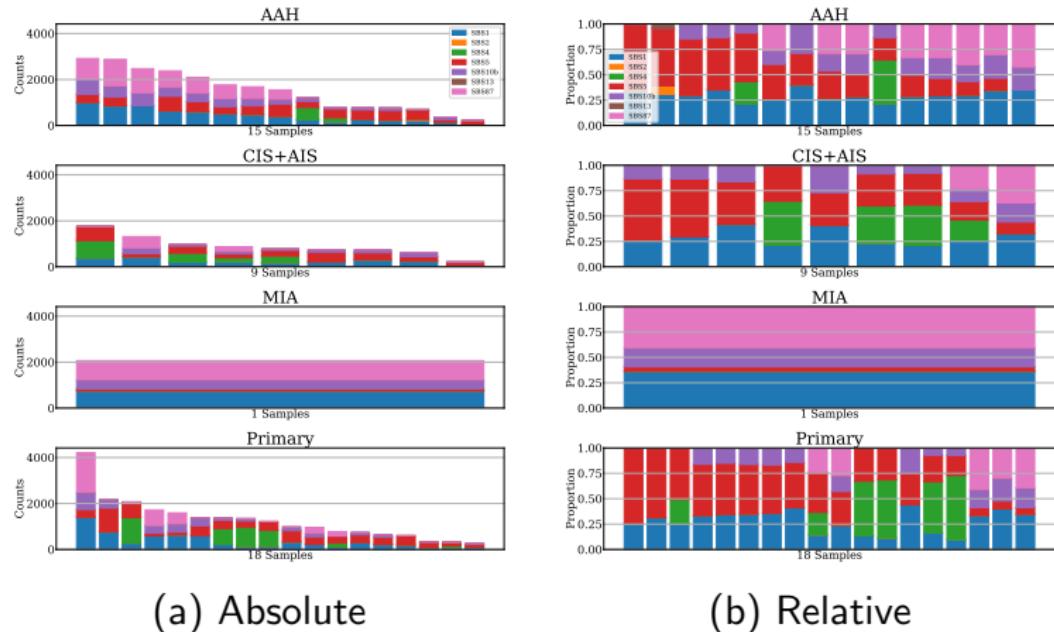


Figure: SBS Bar Plot by Cancer Subtype in LUSC

SBS in LUAD with Smoking I

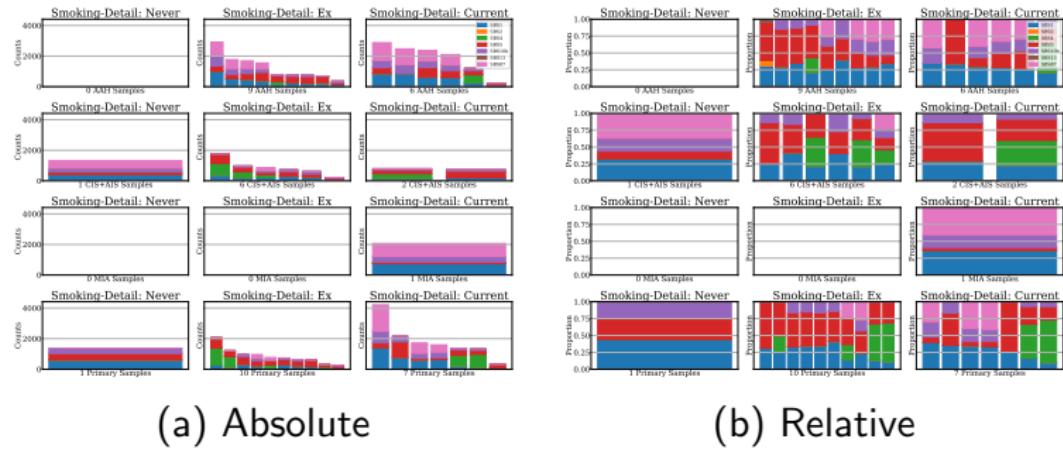
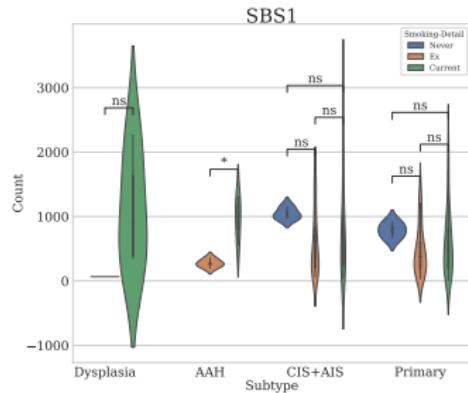
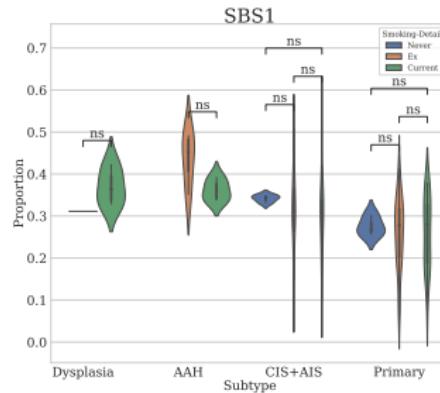


Figure: SBS Bar Plot by Cancer Subtype & Smoking in LUAD

SBS in LUAD with Smoking II



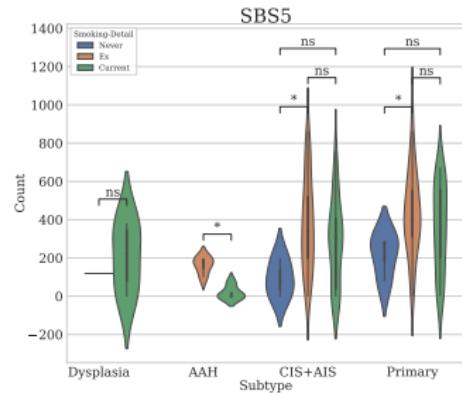
(a) Absolute



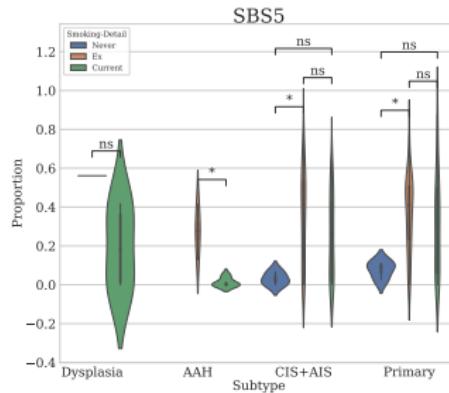
(b) Relative

Figure: SBS1 Signature in LUAD with Smoking

SBS in LUAD with Smoking III



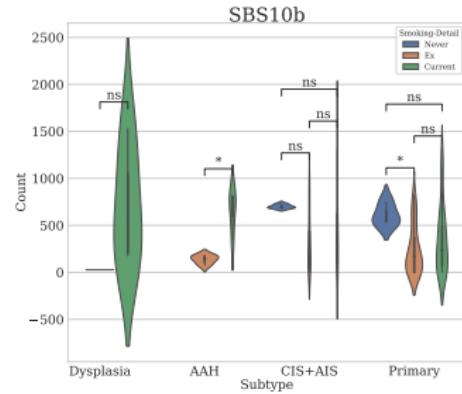
(a) Absolute



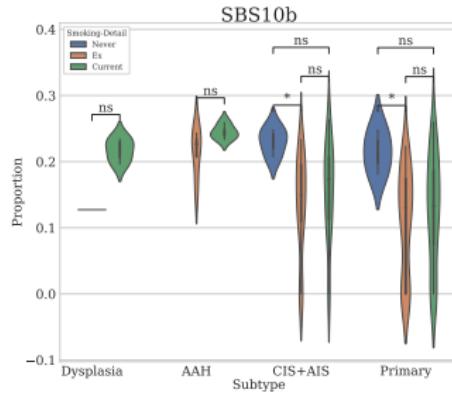
(b) Relative

Figure: SBS5 Signature in LUAD with Smoking

SBS in LUAD with Smoking IV



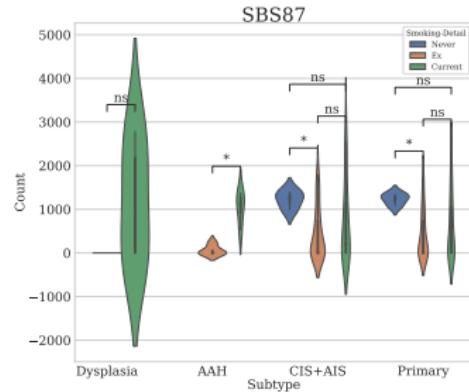
(a) Absolute



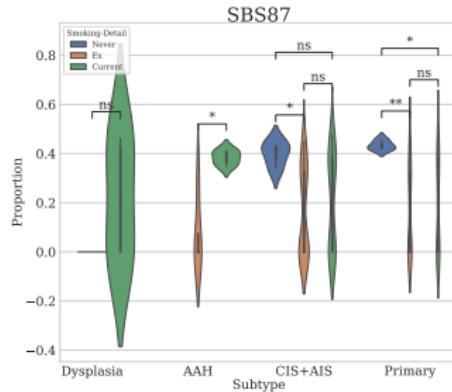
(b) Relative

Figure: SBS10b Signature in LUAD with Smoking

SBS in LUAD with Smoking V



(a) Absolute



(b) Relative

Figure: SBS87 Signature in LUAD with Smoking

SBS in LUAD with Recurrence I

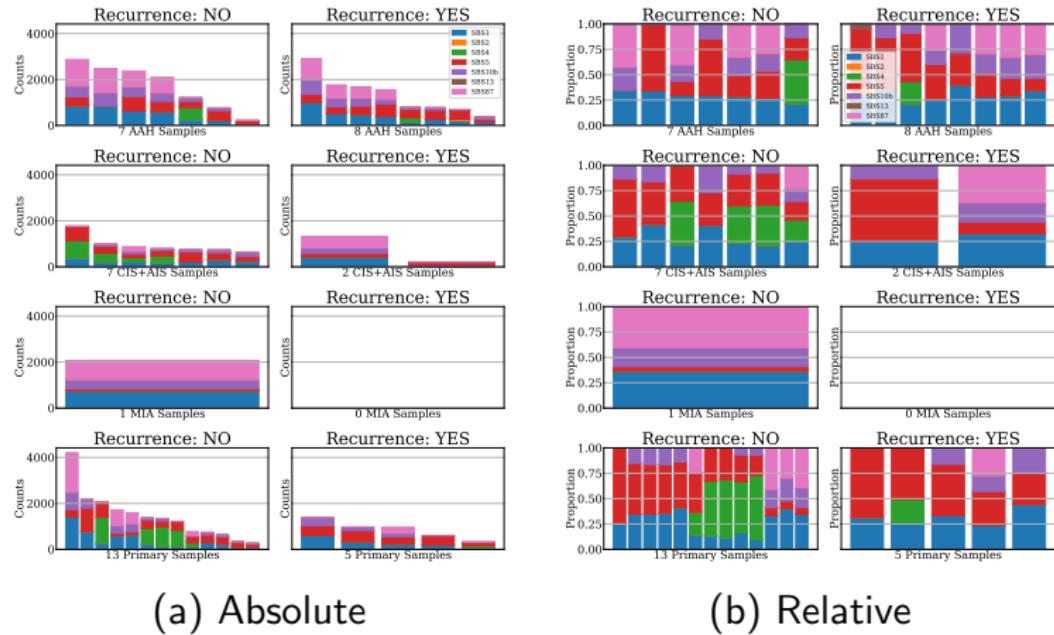
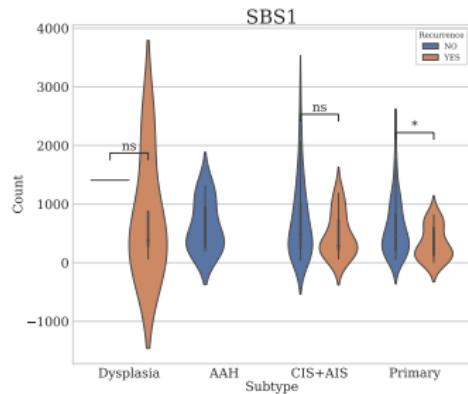
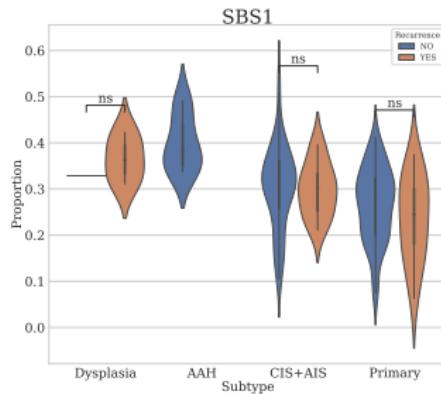


Figure: SBS Bar Plot by Cancer Subtype & Recurrence in LUAD

SBS in LUAD with Recurrence II



(a) Absolute



(b) Relative

Figure: SBS1 Signature in LUAD with Recurrence

4. Results

4.10. Discovery of Mutational Signature

4.10.2. Double Base Substitutions (DBS)

DBS Signatures I

DBS2

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

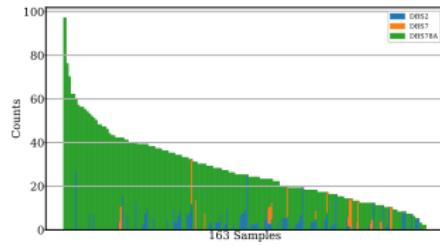
DBS7

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

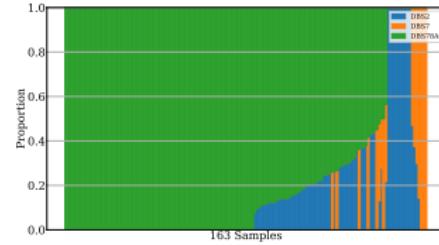
DBS78A

content...

DBS in LUSC I



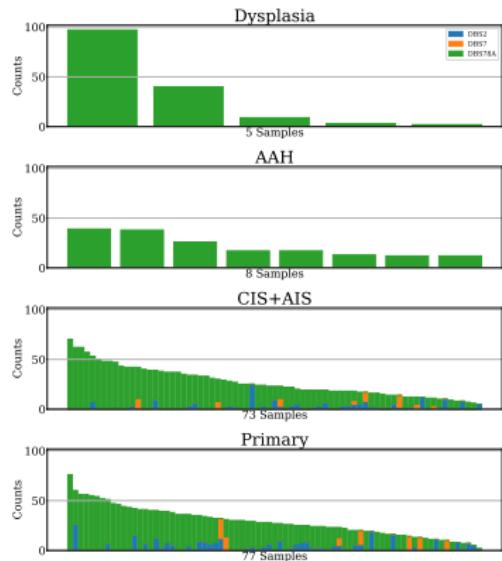
(a) Absolute



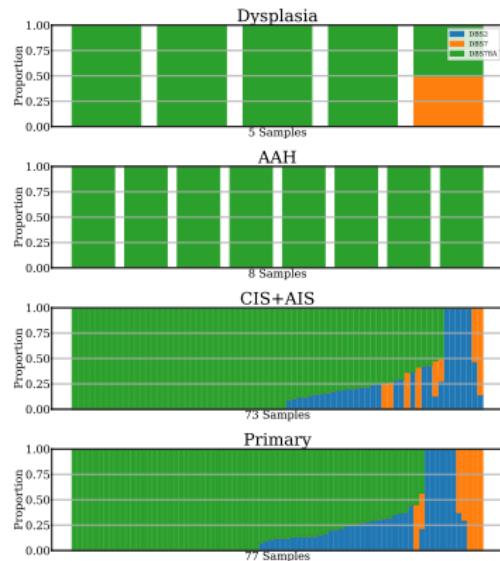
(b) Relative

Figure: DBS Bar Plot in LUSC

DBS in LUSC II



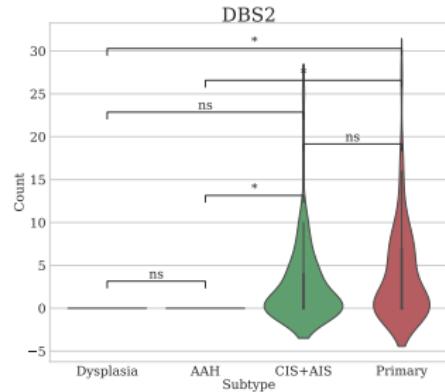
(a) Absolute



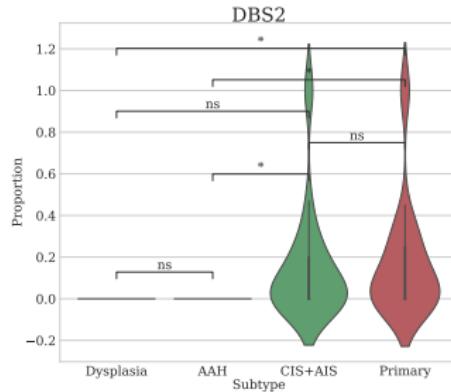
(b) Relative

Figure: DBS Bar Plot by Cancer Subtype in LUSC

DBS in LUSC III



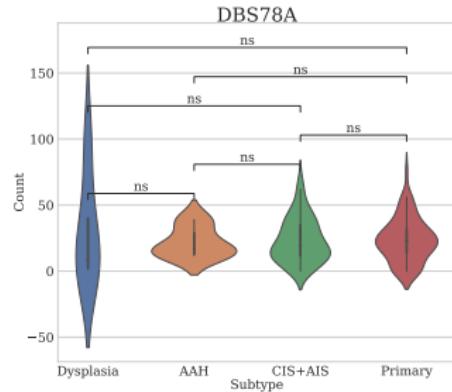
(a) Absolute



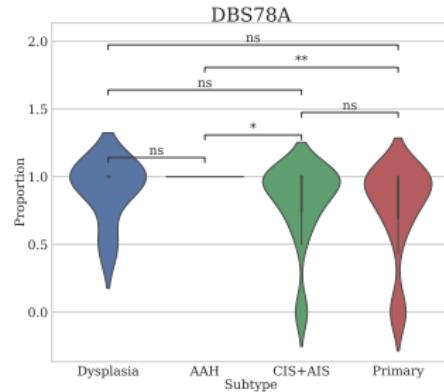
(b) Relative

Figure: DBS2 Signature in LUSC

DBS in LUSC IV



(a) Absolute



(b) Relative

Figure: DBS78A Signature in LUSC

DBS in LUSC with Smoking I

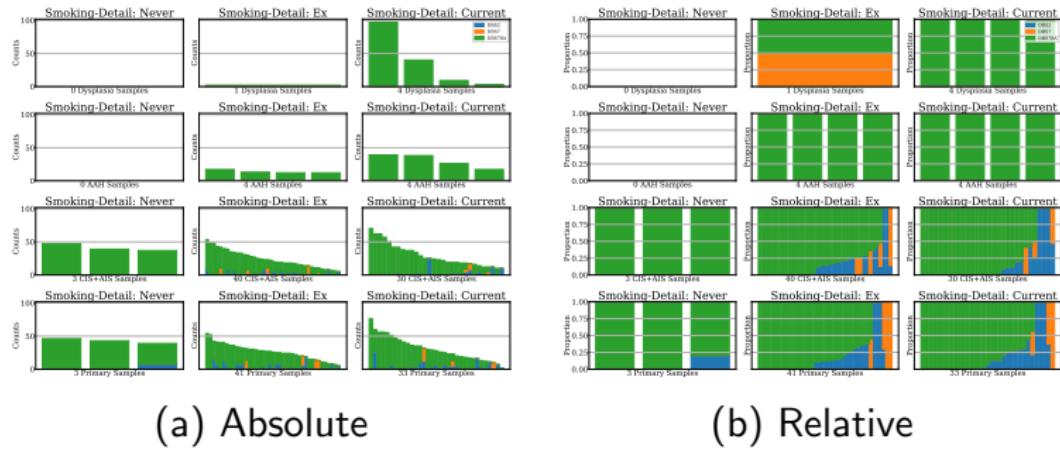
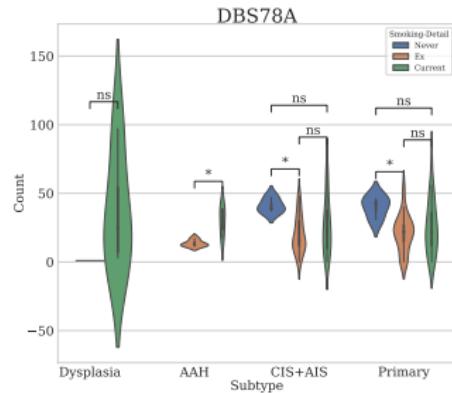
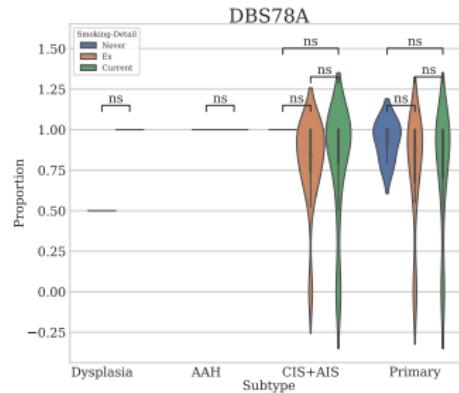


Figure: DBS Bar Plot by Cancer Subtype & Smoking in LUSC

DBS in LUSC with Smoking II



(a) Absolute



(b) Relative

Figure: DBS78A Signature in LUSC with Smoking

DBS in LUSC with Recurrence

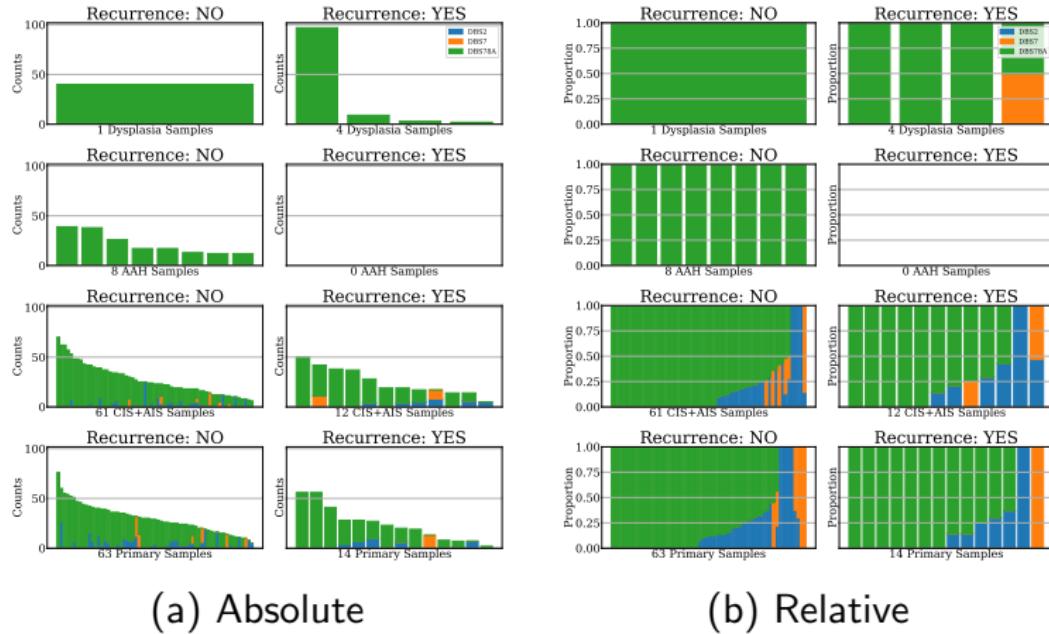
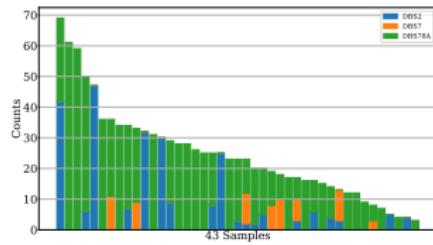
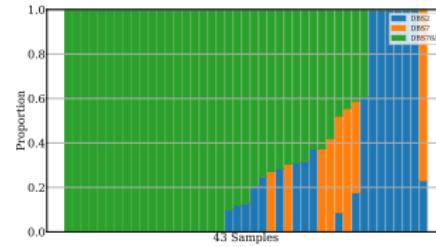


Figure: DBS Bar Plot by Cancer Subtype & Recurrence in LUSC

DBS in LUAD I



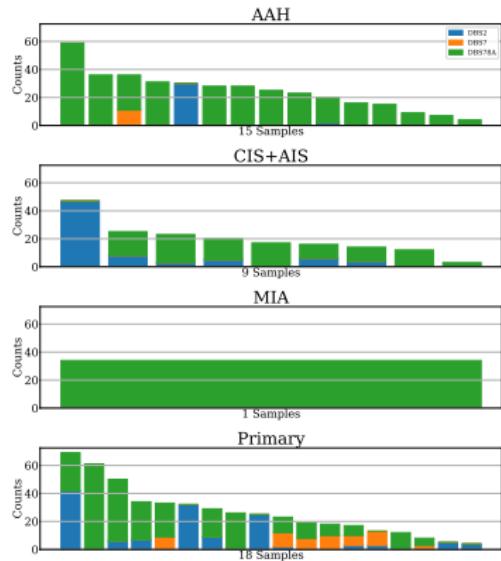
(a) Absolute



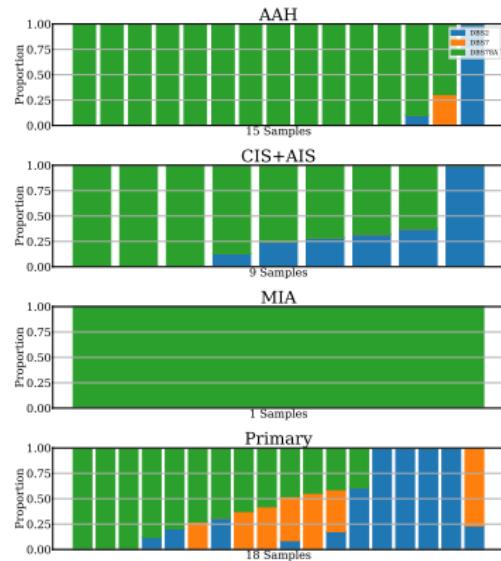
(b) Relative

Figure: DBS Bar Plot in LUAD

DBS in LUAD II



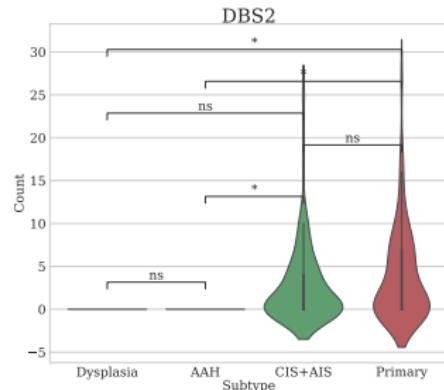
(a) Absolute



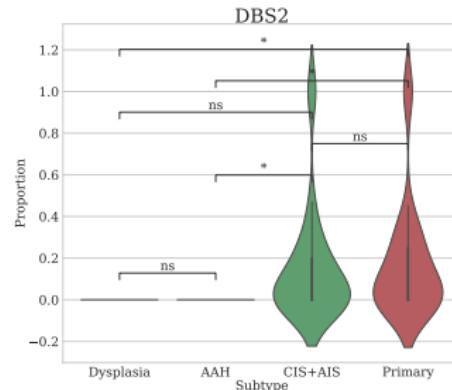
(b) Relative

Figure: DBS Bar Plot by Cancer Subtype in LUAD

DBS in LUAD III



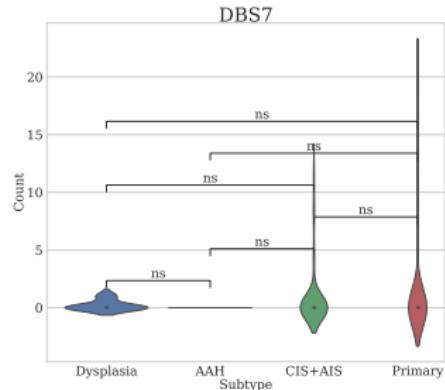
(a) Absolute



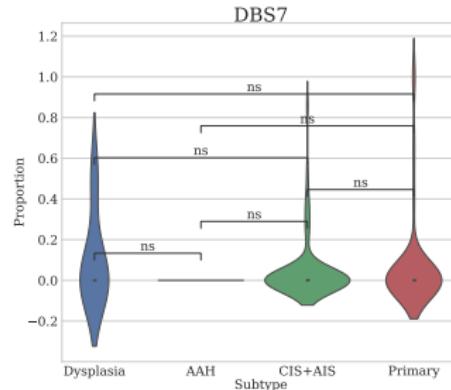
(b) Relative

Figure: DBS2 Signature in LUSC

DBS in LUAD IV



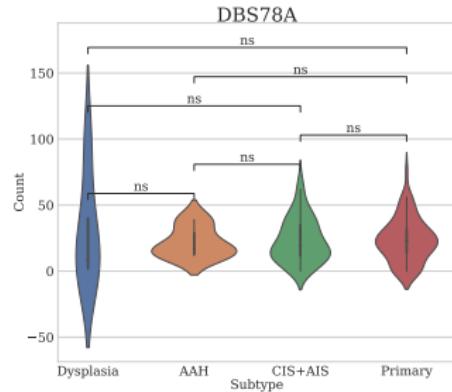
(a) Absolute



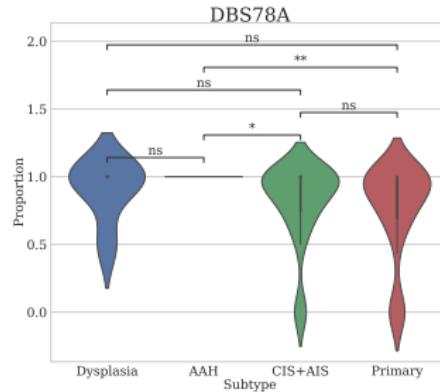
(b) Relative

Figure: DBS7 Signature in LUSC

DBS in LUAD V



(a) Absolute



(b) Relative

Figure: DBS78A Signature in LUSC

DBS in LUAD with Smoking I

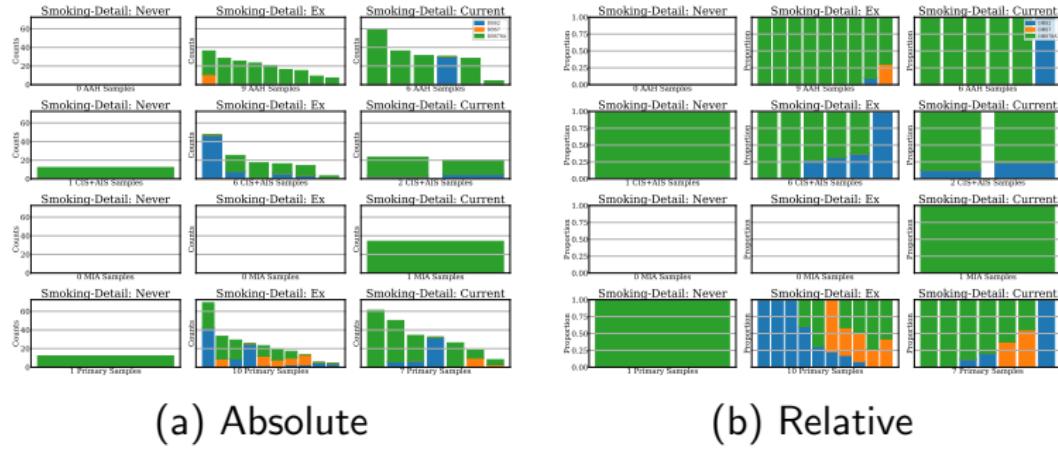
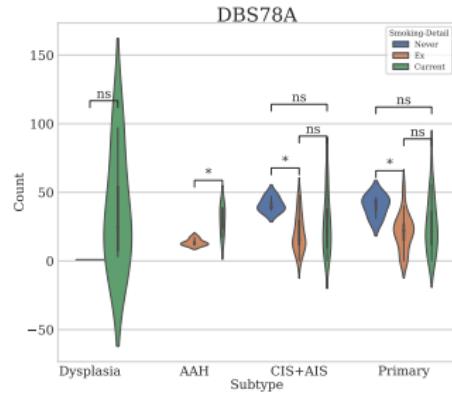
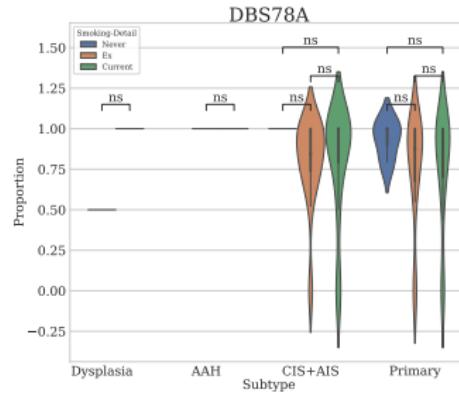


Figure: DBS Bar Plot by Cancer Subtype & Smoking in LUAD

DBS in LUAD with Smoking II



(a) Absolute



(b) Relative

Figure: DBS78A Signature in LUSC in Smoking

DBS in LUAD with Recurrence

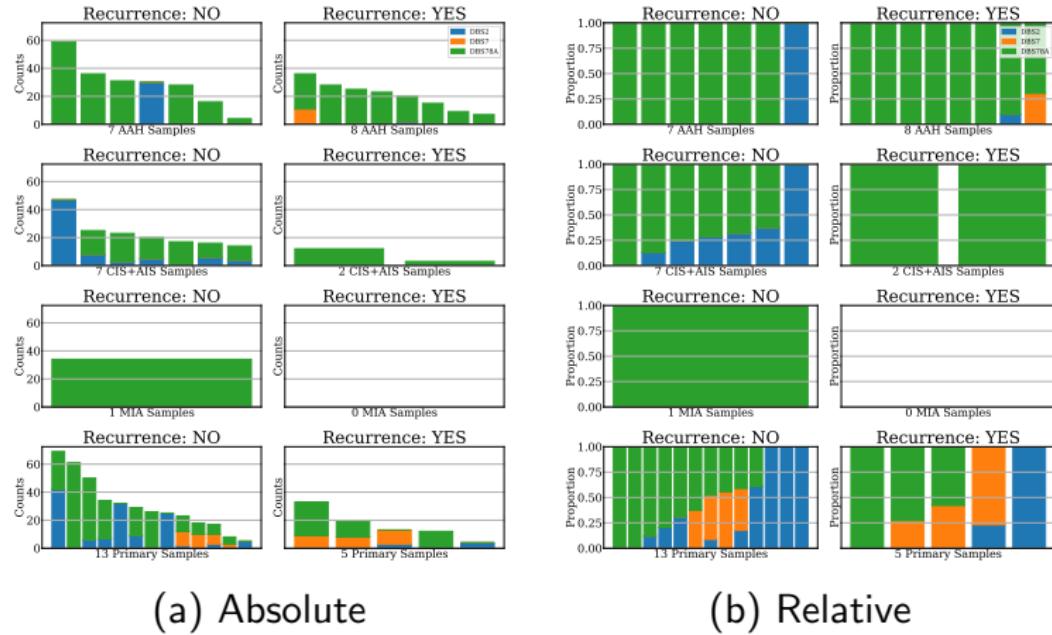


Figure: DBS Bar Plot by Cancer Subtype & Recurrence in LUAD

4. Results

4.10. Discovery of Mutational Signature

4.10.3. Short insertions & Deletions (Indels)

Indel signatures I

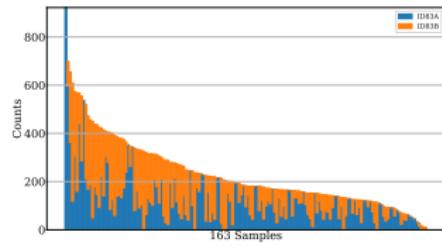
ID83A

content...

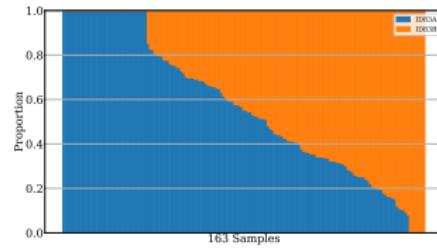
ID83B

content...

Indels in LUSC I



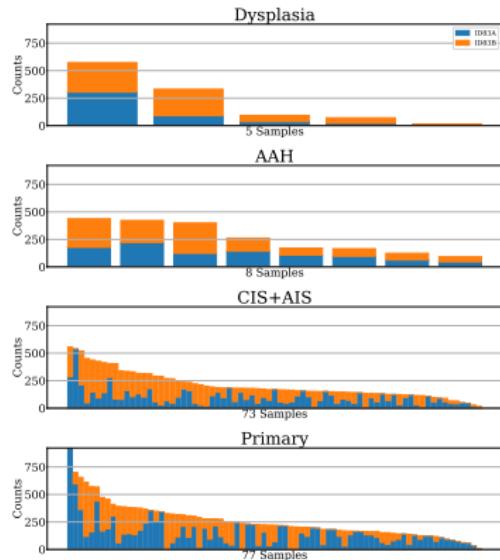
(a) Absolute



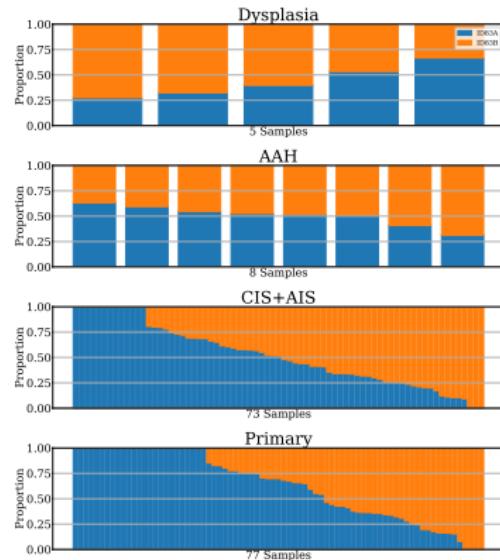
(b) Relative

Figure: Indel Bar Plot in LUSC

Indels in LUSC II



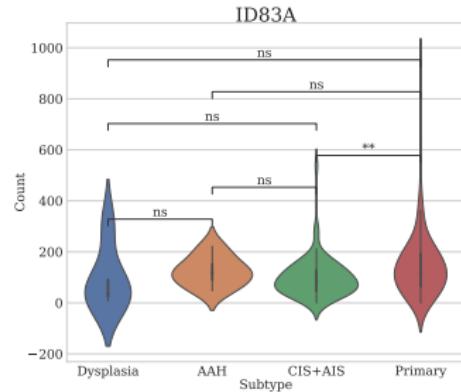
(a) Absolute



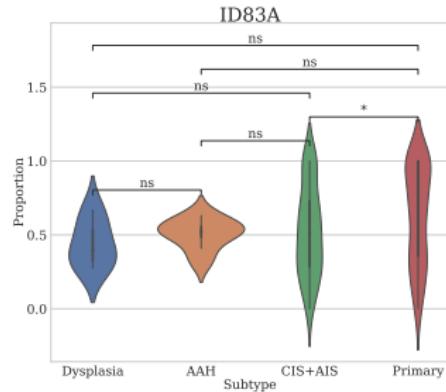
(b) Relative

Figure: Indel Bar Plot by Cancer Subtype in LUSC

Indels in LUSC III



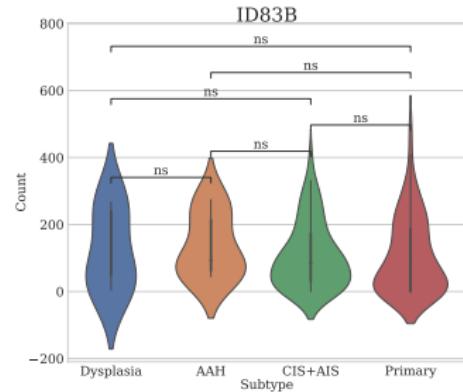
(a) Absolute



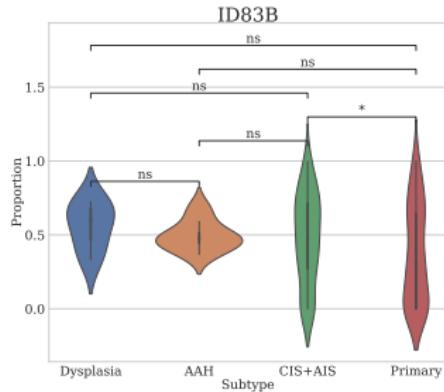
(b) Relative

Figure: Indel83A Signature in LUSC

Indels in LUSC IV



(a) Absolute



(b) Relative

Figure: Indel83B Signature in LUSC

Indel in LUSC with Smoking I

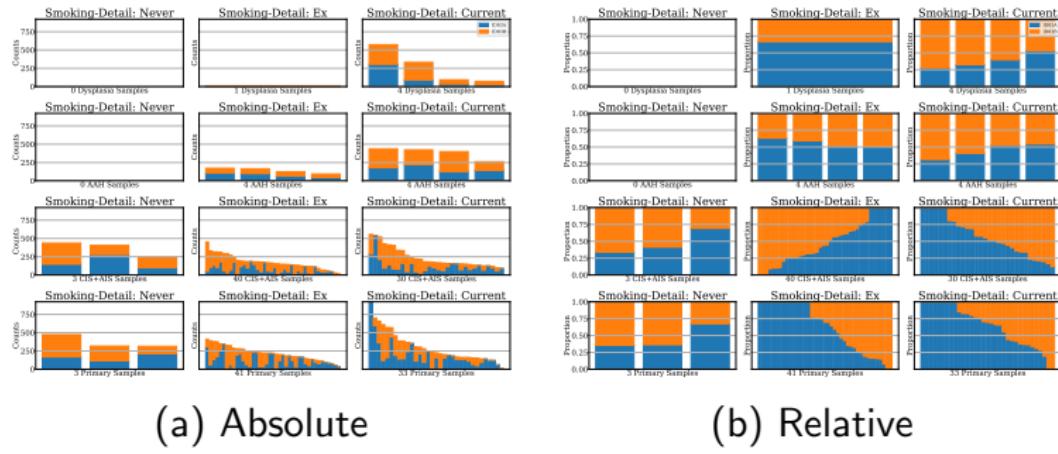
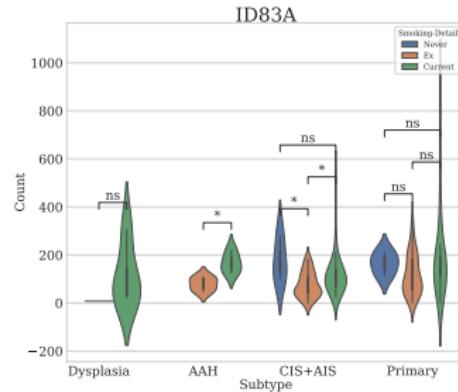
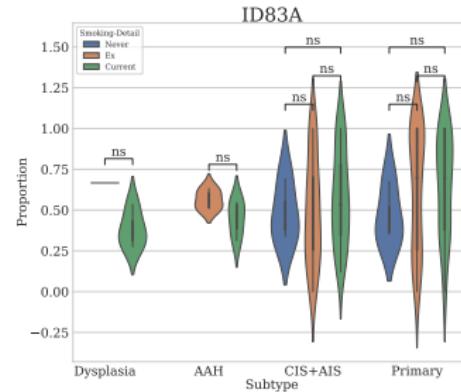


Figure: Indel Bar Plot by Cancer Subtype & Smoking in LUSC

Indel in LUSC with Smoking II



(a) Absolute



(b) Relative

Figure: Indel83A Signature in LUSC with Smoking

Indel in LUSC with Recurrence

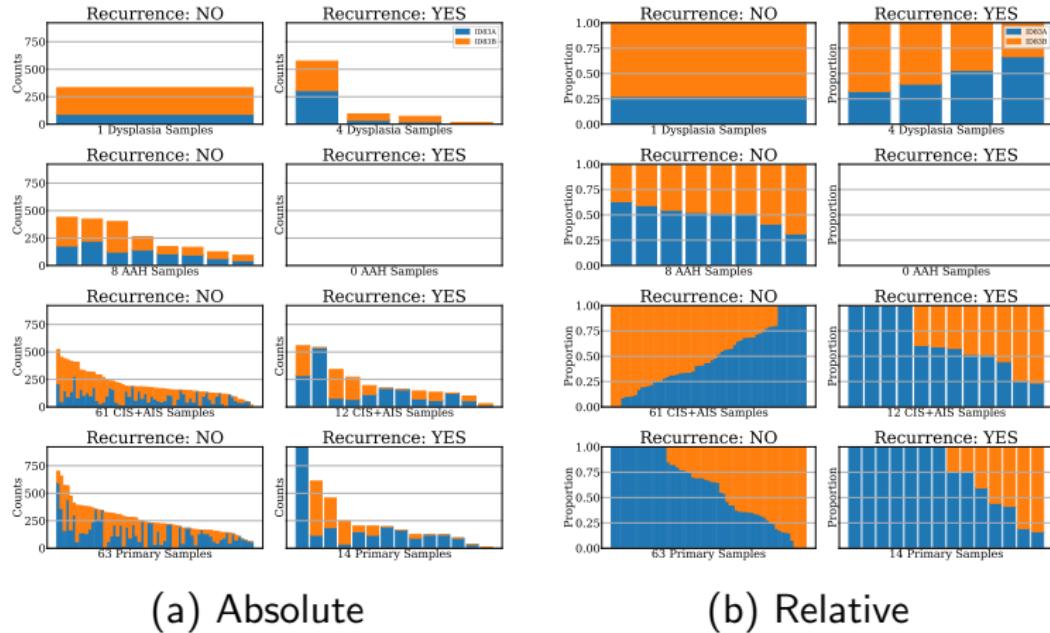
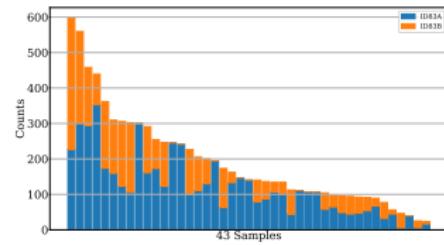
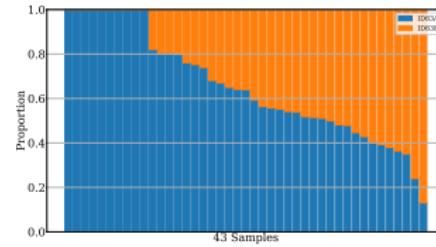


Figure: Indel Bar Plot by Cancer Subtype & Recurrence in LUSC

Indels in LUAD I



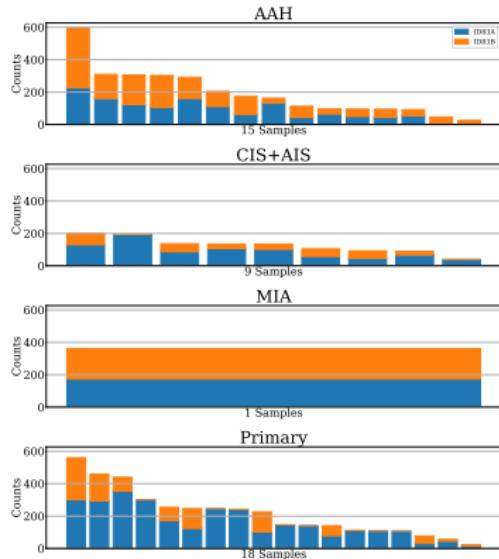
(a) Absolute



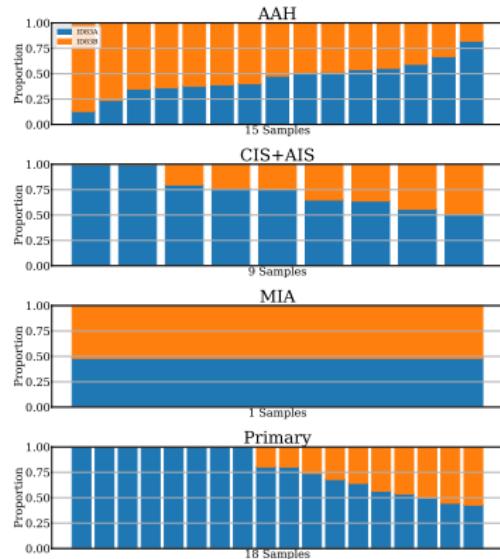
(b) Relative

Figure: Indel Bar Plot in LUAD

Indels in LUAD II



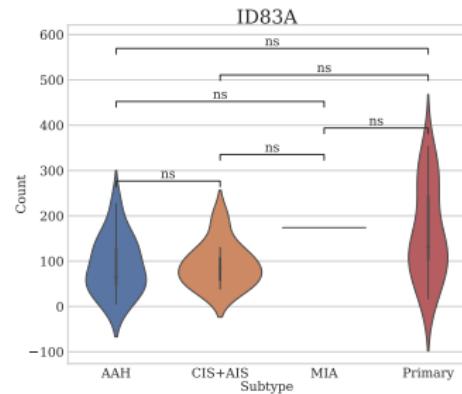
(a) Absolute



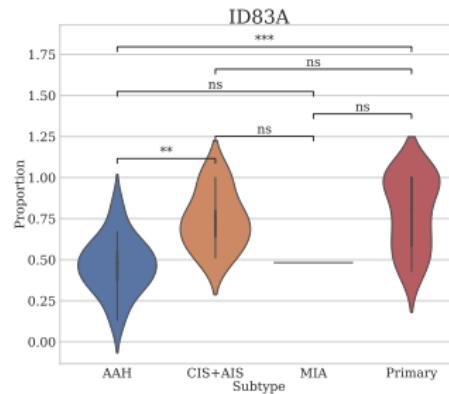
(b) Relative

Figure: Indel Bar Plot by Cancer Subtype in LUAD

Indels in LUAD III



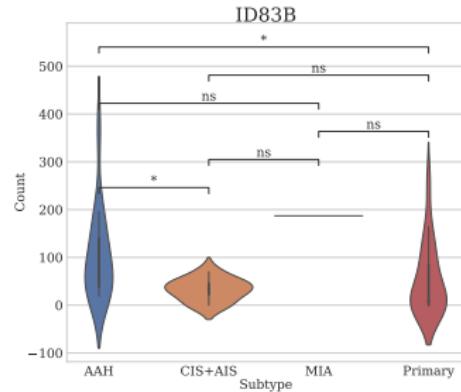
(a) Absolute



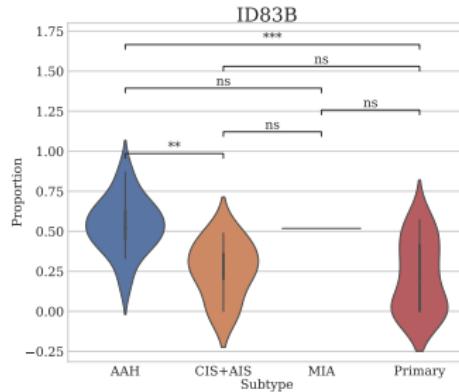
(b) Relative

Figure: Indel83A Signature in LUAD

Indels in LUAD IV



(a) Absolute



(b) Relative

Figure: Indel83B Signature in LUAD

Indel in LUAD with Smoking

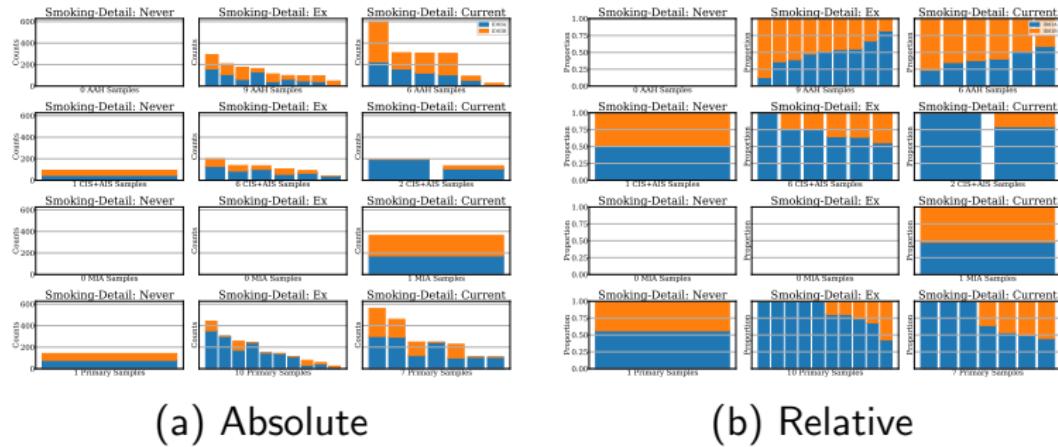


Figure: Indel Bar Plot by Cancer Subtype & Smoking in LUAD

Indel in LUAD with Recurrence

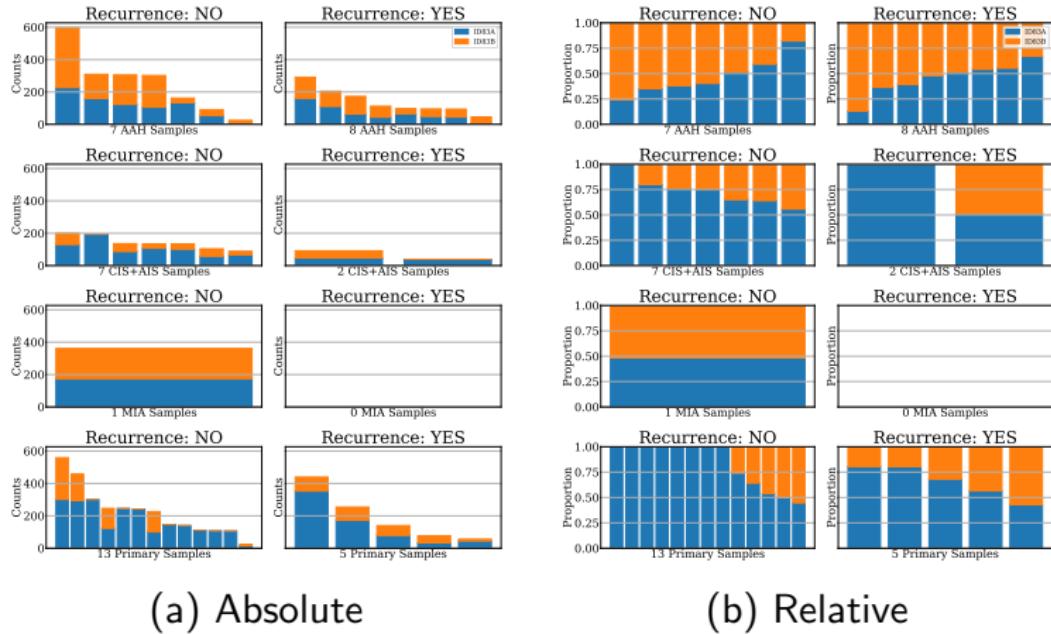


Figure: Indel Bar Plot by Cancer Subtype & Recurrence in LUAD

Findings in Mutation Signature

4. Results

4.11. Clinical Data with Point Mutation

Mutect2?

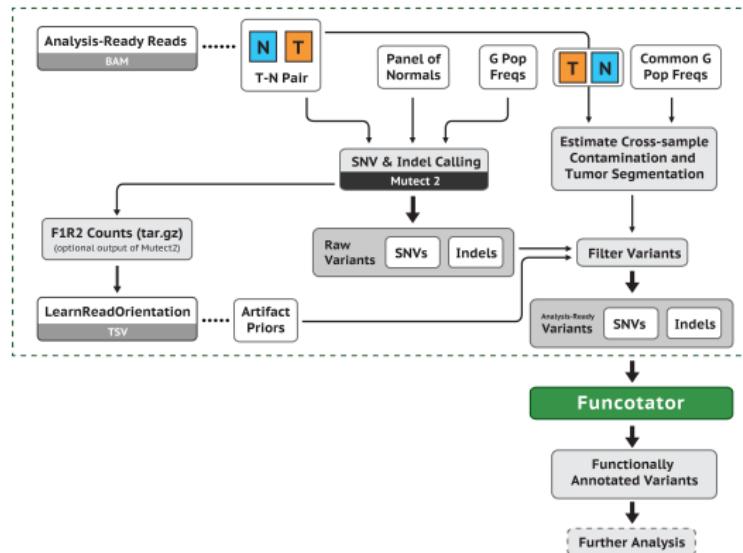


Figure: Somatic short variant discovery workflow (Van der Auwera et al., 2013; DePristo et al., 2011)

4. Results

4.11. Clinical Data with Point Mutation

4.11.1. For Smoking

LUSC with Smoking

Table: LUSC WES Data with Smoking

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

Clinical Data about LUSC for Smoking I

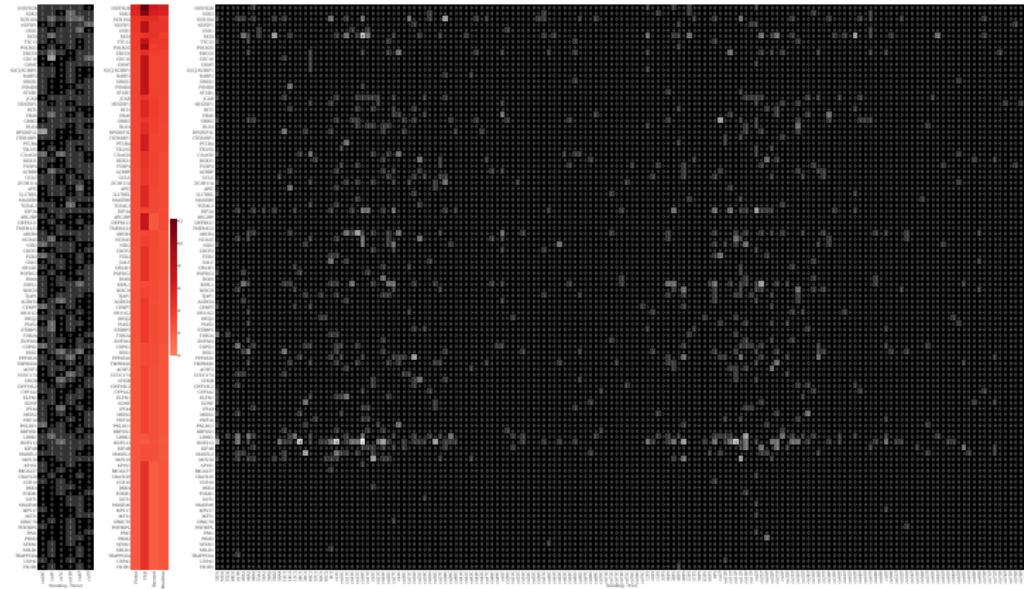


Figure: Clinical Data about LUSC for Smoking

Clinical Data about LUSC for Smoking II

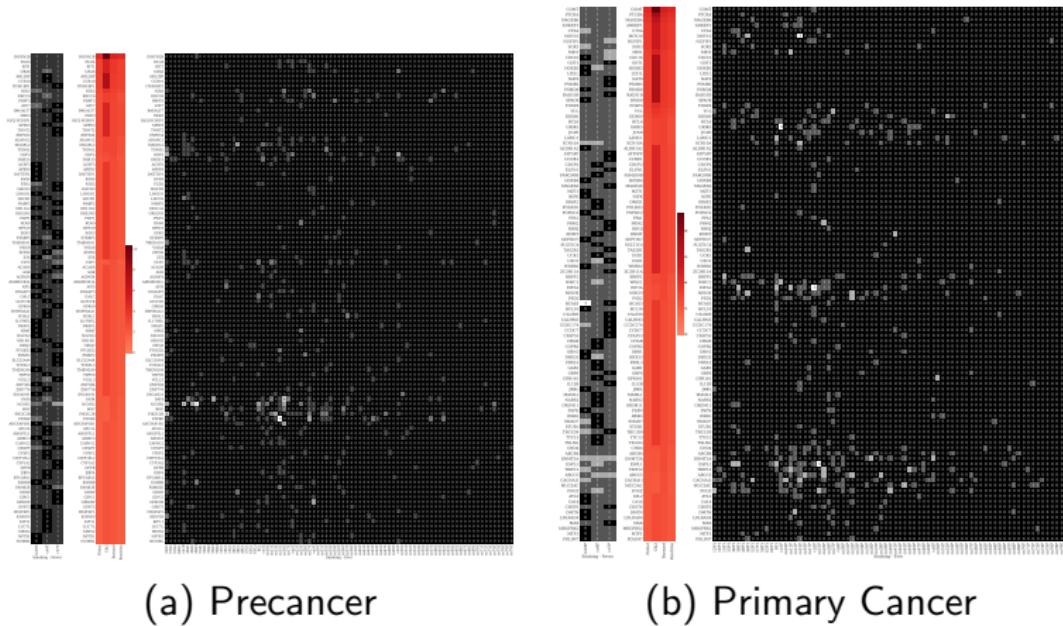


Figure: Clinical Data about LUSC for Smoking with Precancer/Primary

Notable genes in LUSC for Smoking I

INSYN2B

content...

SGK3

content...

SCN10A

content...

INSYN2B

content...

DLG4

content...

Notable genes in LUSC for Smoking II

RIT1

content...

COMT

content...

PTCRA

content...

MAGEB6

content...

LUAD with Smoking

Table: LUAD WES Data with Smoking

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

Clinical Data about LUAD for Smoking I

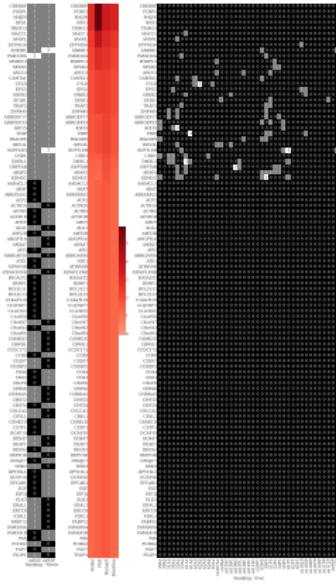
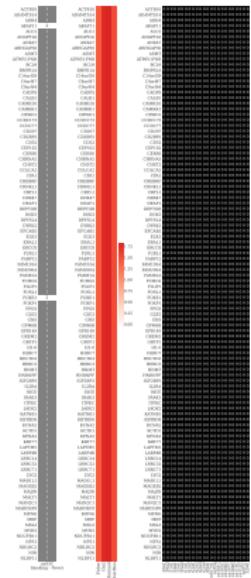
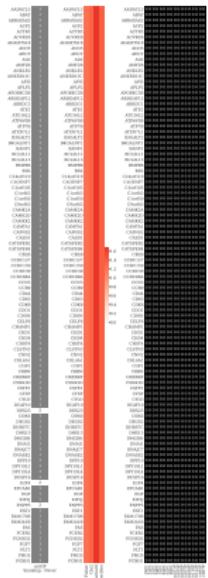


Figure: Clinical Data about LUAD for Smoking

Clinical Data about LUAD for Smoking II



(a) Precancer



(b) Primary Cancer

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

Notable genes in LUAD for Smoking I

CREBRF

content...

FOXP1

content...

MAJIN

content...

ACTR10

content...

ADAMTS14

content...

Notable genes in LUAD for Smoking II

ADH4

content...

AADACL3

content...

ABAT

content...

ABRAXAS2

content...

4. Results

4.11. Clinical Data with Point Mutation

4.11.2. For Recurrence

LUSC with Recurrence

Table: LUSC WES Data with Recurrence

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

Clinical Data about LUSC for Recurrence I

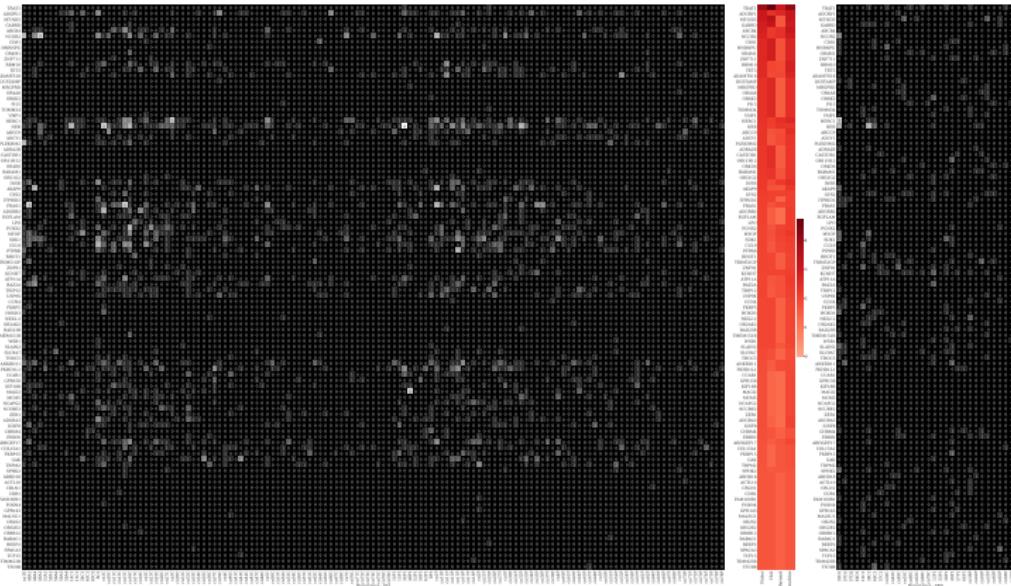


Figure: Clinical Data about LUSC for Recurrence

Clinical Data about LUSC for Recurrence II

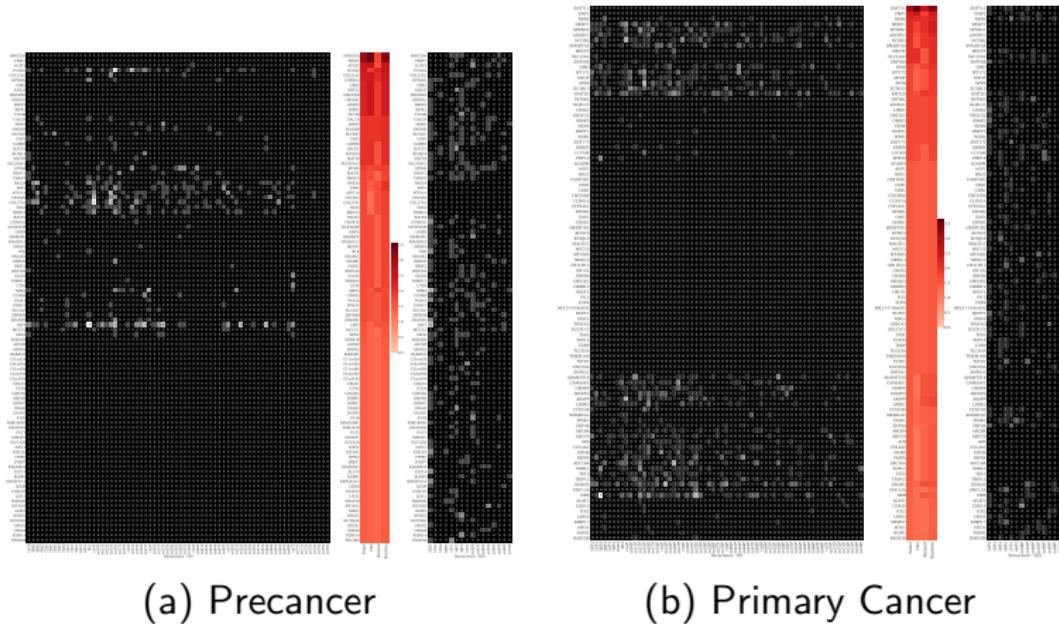


Figure: Clinical Data about LUSC for Recurrence with Precancer/Primary

Notable genes in LUSC with Recurrence I

TRAT1

content...

ADGRV1

content...

MT-ND5

content...

HMG20A

content...

TRAT1

content...

Notable genes in LUSC with Recurrence II

ACAT2

content...

ZNF711

content...

VMP1

content...

RERE

content...

LUAD with Recurrence

Table: LUAD WES Data with Recurrence

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

Clinical Data about LUAD for Recurrence I

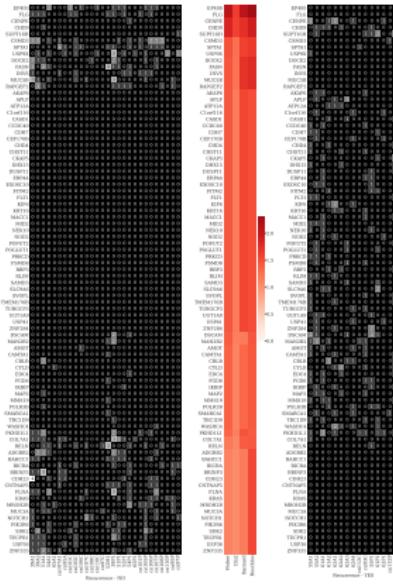
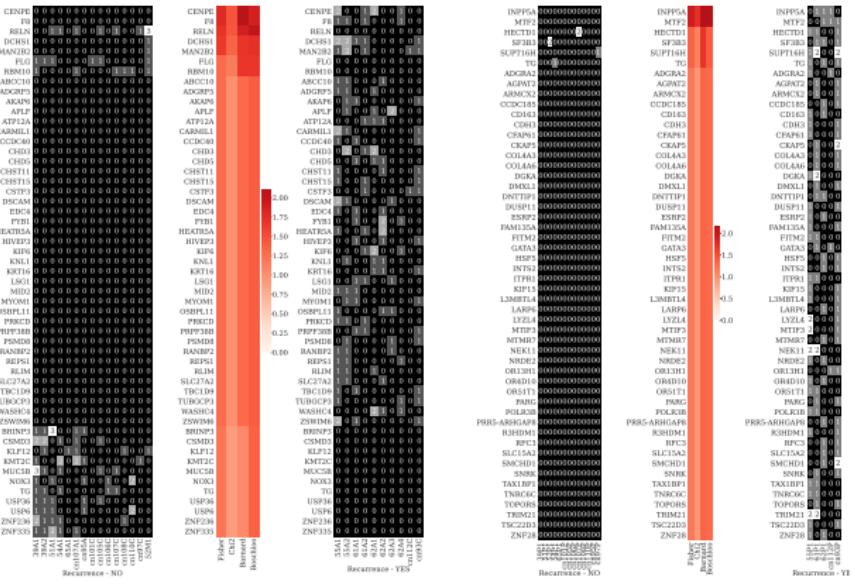


Figure: Clinical Data about LUAD for Recurrence

Clinical Data about LUAD for Recurrence II



(a) Precancer

(b) Primary Cancer

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

Notable genes in LUSC with Recurrence I

EP400

content...

FLG

content...

CENPE

content...

CENPE

content...

F8

content...

Notable genes in LUSC with Recurrence II

RELN

content...

INPP5A

content...

MTF2

content...

HECTD1

content...

Findings in Clinical Data with Point Mutation

5. Discussion

6. References

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