

Lung Precancer Analysis

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Overview

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1. Introduction

1. Introduction

1.1. Lung Cancer

Lung Cancer?

The most common cancer

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

The most important factor

Tobacco

Cancer Survival Rate in Korea

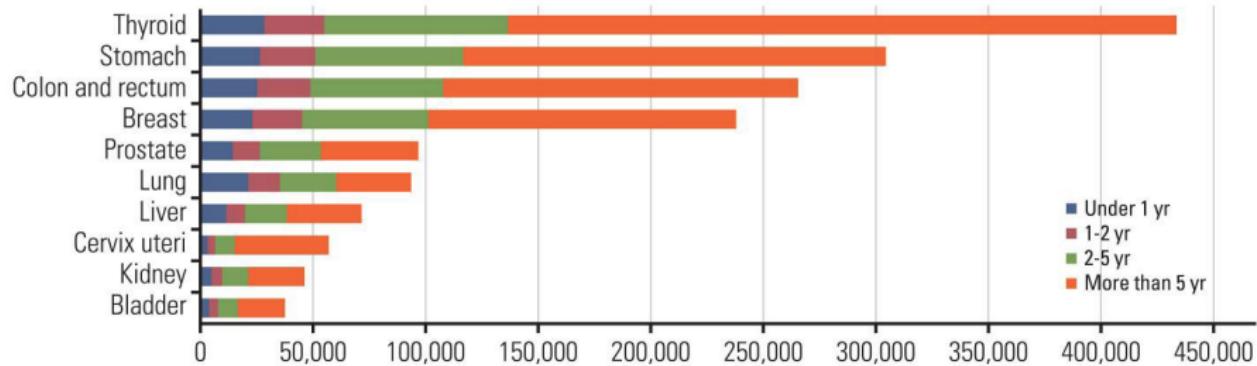


Figure: Common cancer survival rates (S. Hong et al., 2021)

Survival rate (More than 5 year)

- Thyroid: 68.4 %
- Lung: 35.4 %

Type of Lung Cancer I

Types of lung cancer (Collins, Haines, Perkel, & Enck, 2007):

- 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)
- ⑧ ...

Type of Lung Cancer II

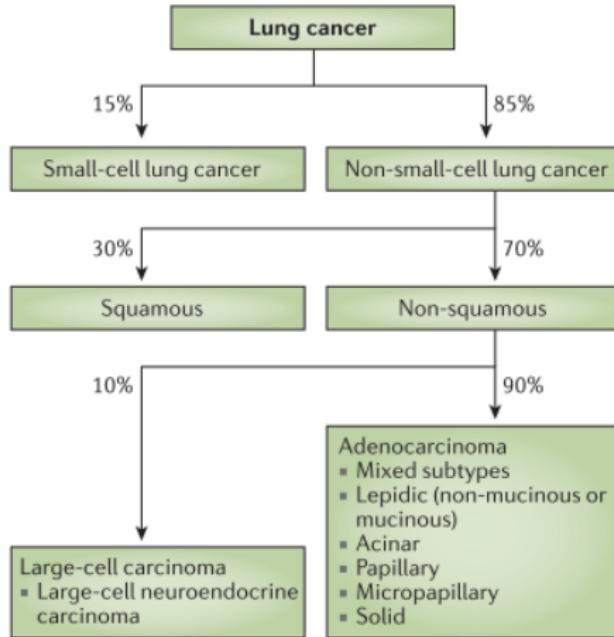


Figure: Lung cancer classification (Gridelli et al., 2015)

1. Introduction

1.2. Non-small cell lung cancer

Non-small cell lung cancer (NSCLC)

Types of NSCLC (Goldstraw et al., 2011):

- Adenocarcinoma (ICDO 8140/3)
- Squamous cell carcinoma (ICDO 8070/3)
- Large-cell carcinoma (ICDO 8012/3)
- ...

1. Introduction

1.3. LUAD

TCGA LUAD (Duhig et al., 2014)

- 81 % patients reported past/present tobacco smoking.
- Candidate driver genes: RTK, RAS, and RAF (38 %)
- Cancer-associated mutations: KRAS (32 %), EGFR (11 %), and BRAF (7 %)
- Enriched mutations: TP53, KEAP1, NF1, and RIT1 ($p < 0.01$)
- Fusions: ROS1 and RET

1. Introduction

1.4. LUSC

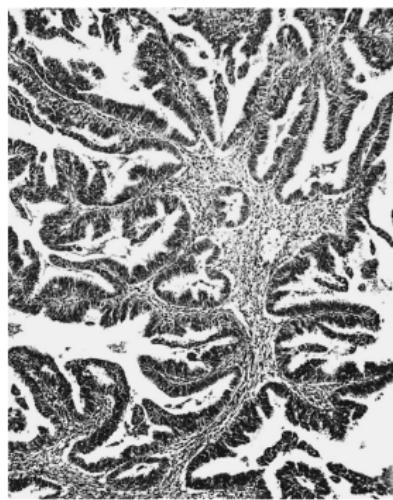
TCGA LUSC (Dosenbach et al., 2007)

- 96 % patients reported past/present tobacco smoking.
- Not present EGFR and ALK fusions.
- Recurrent mutations: TP53, NFE2L2, KEAP1, BAI3, FBXW7, GRM8, MUC16, RUNX1T1, STK11, and ERBB4
- High rate of copy number alteration compared with other TCGA projects.
- Amplification of NFE2L2, MYC, CDK6, MDM2, BCL2L1, and EYS.
- Deletion of FOXP1, PTEN, and NF1

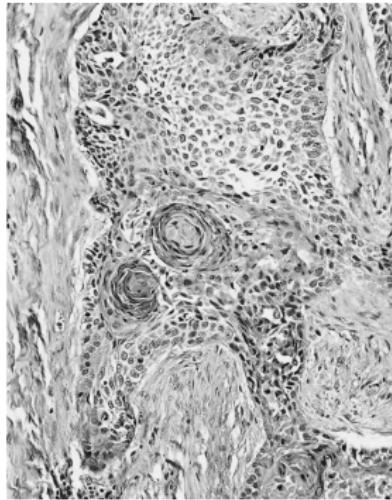
1. Introduction

1.5. LUAD vs. LUSC

LUAD vs. LUSC I



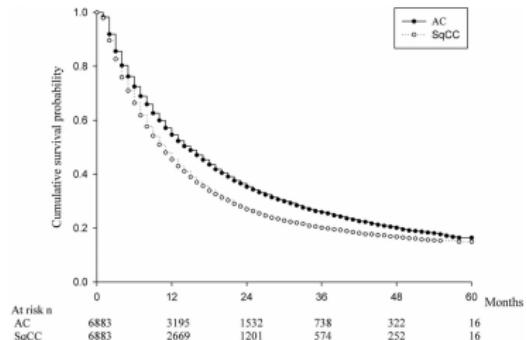
(a) LUAD



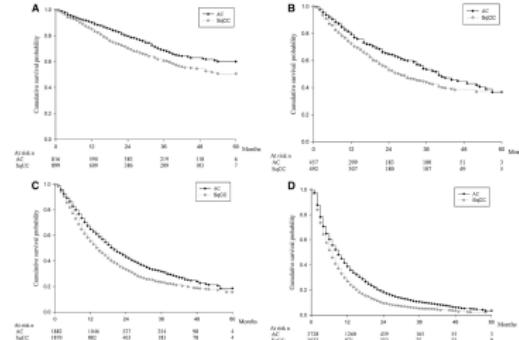
(b) LUSC

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

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) (sample n=289) + Transcriptome (WTS) (sample n=166)
- Normal + {Dysplasia, AAH, CIS + AIS, MIA} + Primary
 - Adenocarcinoma *in situ*
 - Atypical adenomatous hyperplasia
 - Carcinoma *in situ*
 - Dysplasia
 - Minimally invasive adenocarcinoma
- Adenocarcinoma (LUAD) & Squamous cell carcinoma (LUSC)
 - ① Normal → AAH → AIS → MIA → LUAD (patient n=28)
 - ② Normal → Dysplasia → CIS → LUSC (patient 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 | 20 | |
| | Primary | | |
| | Total | 13 | 7 |
| Non-recurrence | Normal | 7 | 1 |
| | AAH | 7 | 13 |
| | CIS+AIS | | |
| | MIA | 41 | |
| | Primary | | |
| | Total | 1 | |

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 | Total |
| Recurrence | Normal | 2 | 2 |
| | CIS+AIS | 1 | 1 |
| | Primary | 1 | 1 |
| | Total | 4 | 4 |
| Non-recurrence | Normal | 11 | 11 |
| | AAH | 1 | 1 |
| | CIS+AIS | 4 | 4 |
| | Primary | 5 | 5 |
| | Total | 21 | 21 |

WTS Data Composition with Smoking I

Table: LUSC WTS Data with Smoking

| Smoking? | Stage | Number of Samples | |
|----------|-----------|-------------------|-----|
| | | Normal | 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

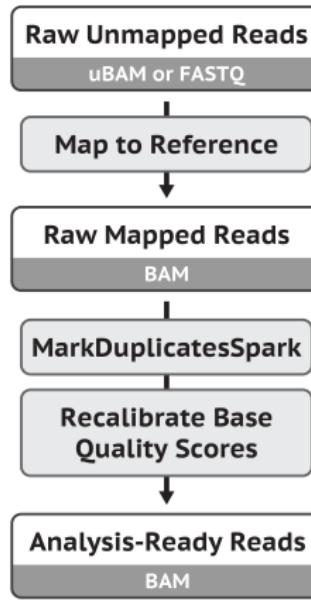


Figure: Data pre-processing for variant discovery (Van der Auwera et al., 2013; DePristo et al., 2011)

Somatic short variant discovery

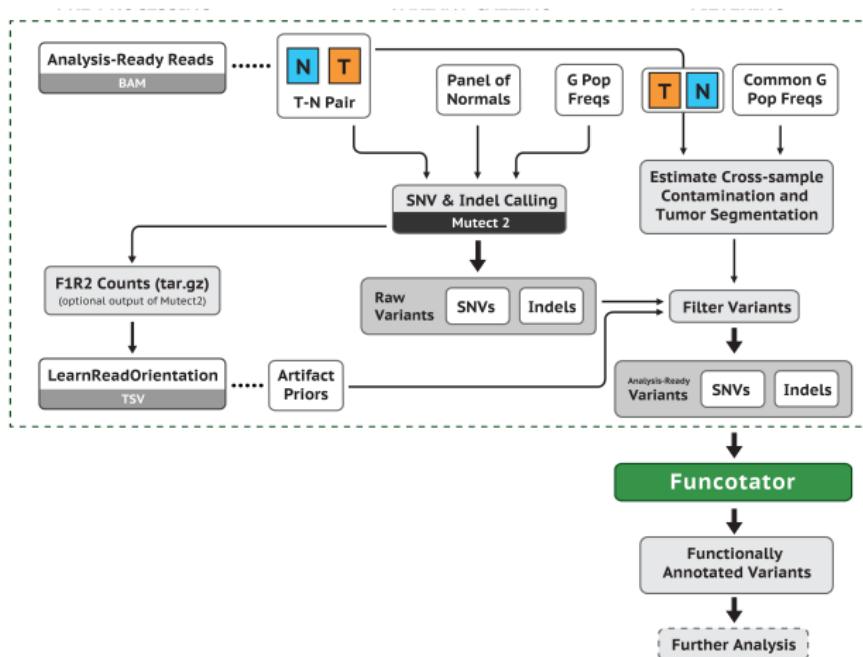


Figure: Somatic short variant (SNVs + Indels) discovery workflow (Van der Auwera et al., 2013; DePristo et al., 2011)

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

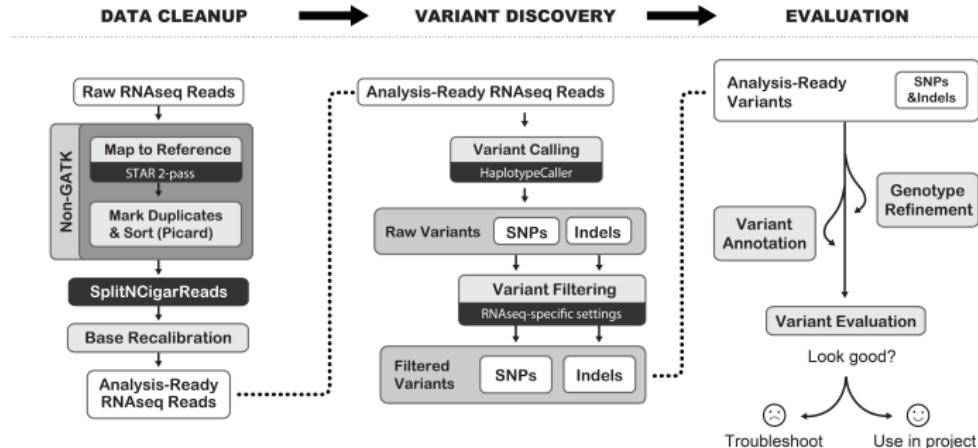


Figure: RNA-seq short variant (SNVs + Indels) discovery workflow (Van der Auwera et al., 2013; DePristo et al., 2011)

4. Results

4. Results

4.1. Quality Checks with FastQC

FastQC?

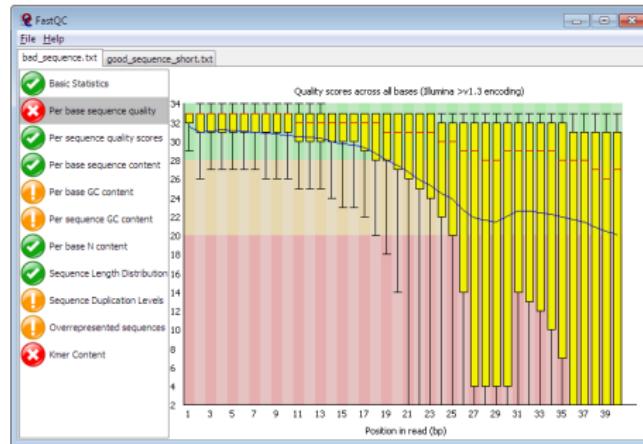


Figure: Example of FastQC Result (Andrews et al., 2012)

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

FastQC on WES

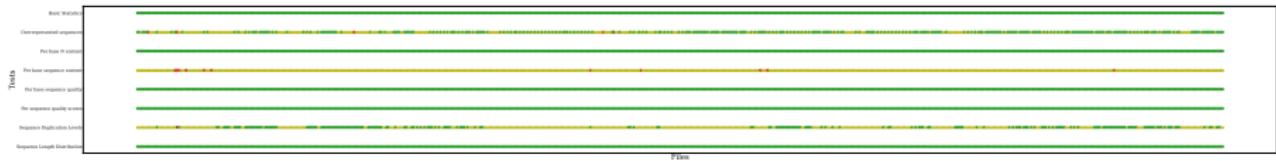
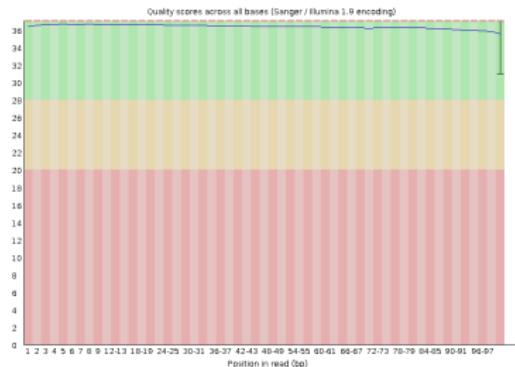


Figure: FastQC with WES data

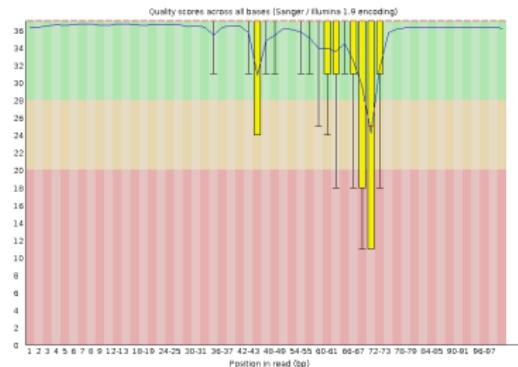
Failure on 33P1 sample

33P1 is excluded from further analyses.

Failure on 33P1 I



(a) 33N



(b) 33P1

Figure: Per Base Sequence Quality Results

Failure on 33P1 II

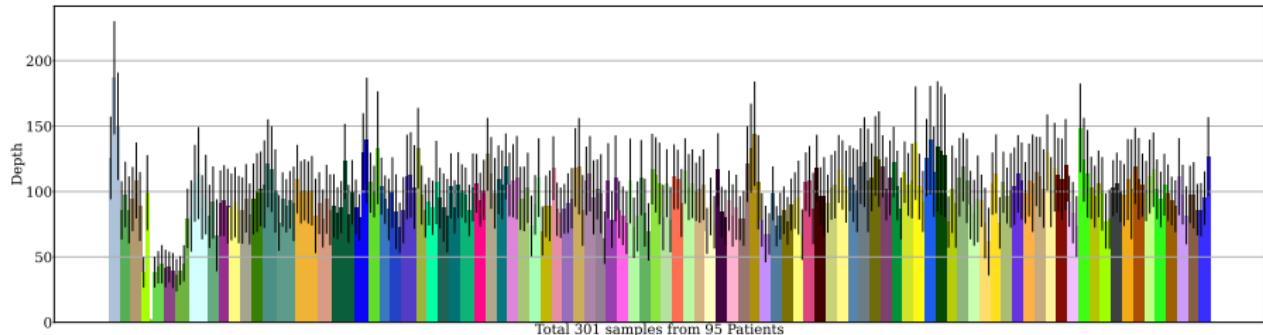


Figure: Coverage Depth Plot

- Tumor DNA: $97.6\times$; Germline DNA: $95.8\times$ in TCGA LUAD (Duhig et al., 2014)
- Mean $121\times$, with 83 % of target bases above $30\times$ in TCGA LUSC (Dosenbach et al., 2007)

FastQC on WTS

Tests

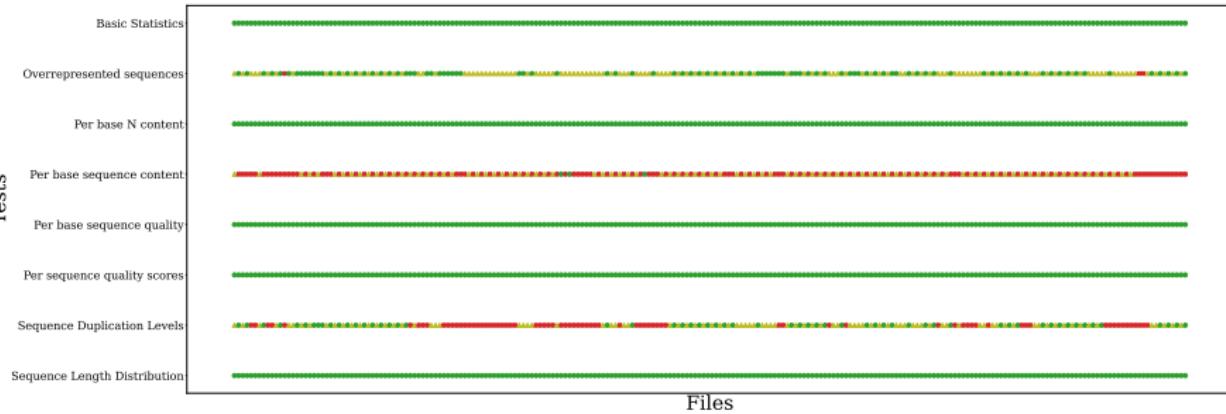


Figure: FastQC with WTS data

All sample are good to analysis

∴ No sample has more than 5 failures.

4. Results

4.2. Quality Checks with Picard

Picard?

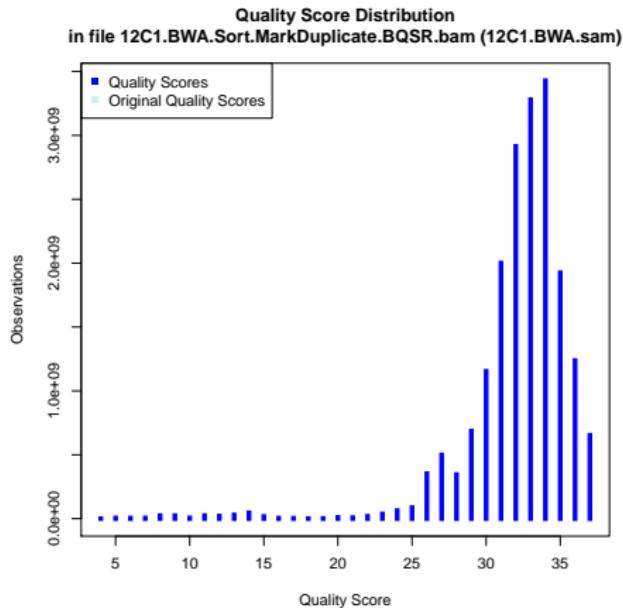


Figure: Quality Distribution of 12C1 sample

Quality Distribution Plot

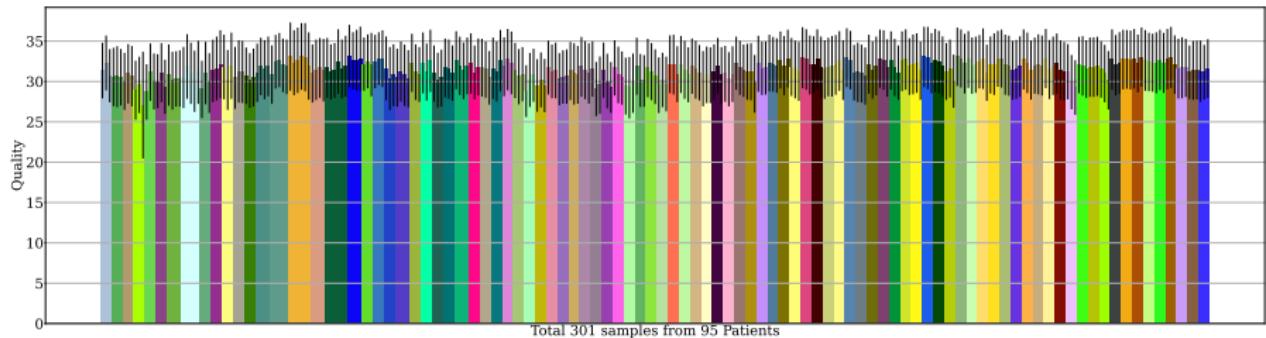


Figure: Quality Distribution by Samples

Findings in Picard

4. Results

4.3. Copy Number Variations

PureCN?

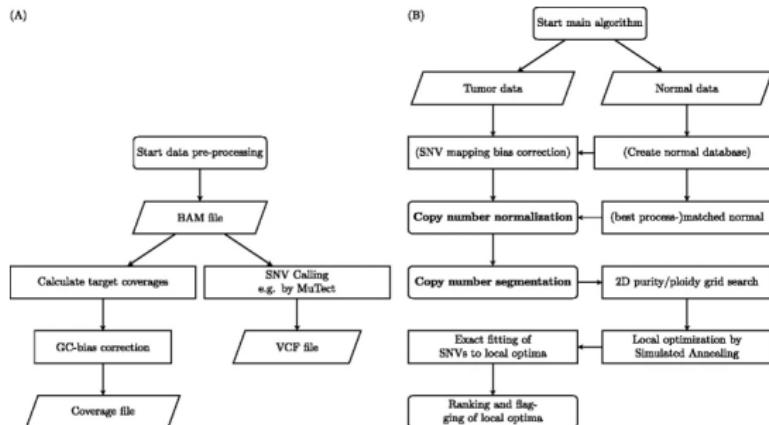
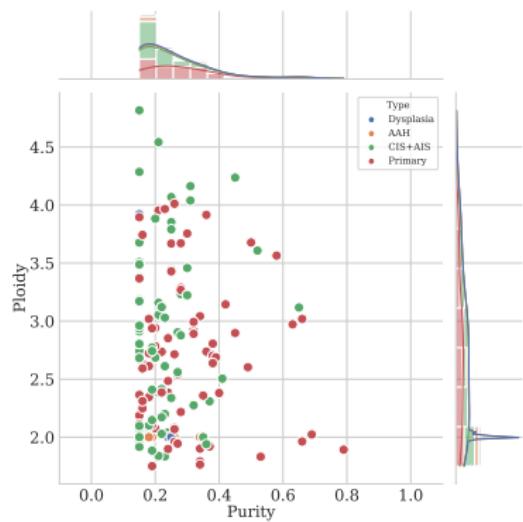
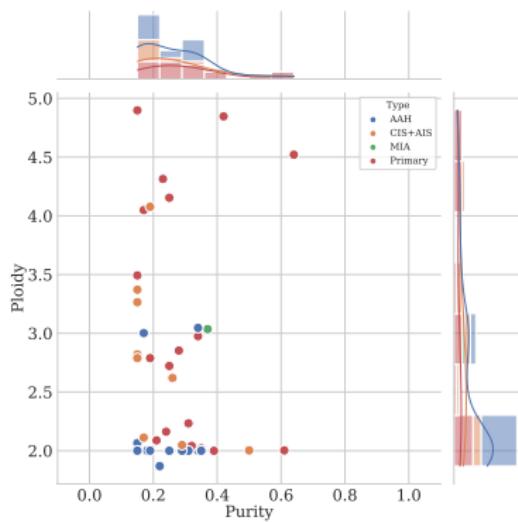


Figure: Flowchart of the PureCN data pre-processing pipeline (Riester et al., 2016)

Purity & Ploidy on WES



(a) LUSC Samples



(b) LUAD Samples

Figure: Cellularity and Ploidy from PureCN

LUSC in CNV Plot I

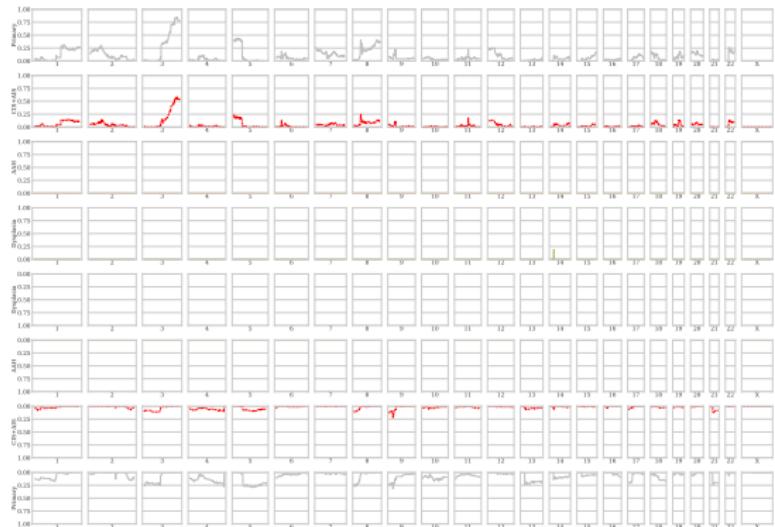


Figure: LUSC in CNV Plot

LUSC in CNV Plot II



Figure: LUSC in Violin Plots

LUSC with Recurrence in CNV Plot I



Figure: LUSC with Recurrence in CNV Plot

LUSC with Recurrence in CNV Plot II

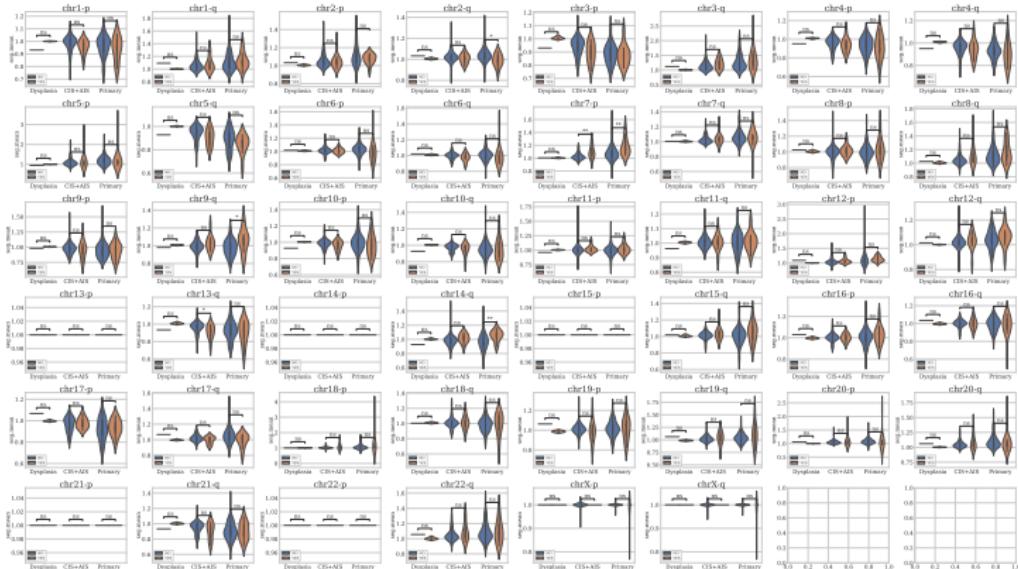


Figure: LUSC with Recurrence in Violin Plots

LUSC with Smoking in CNV Plot

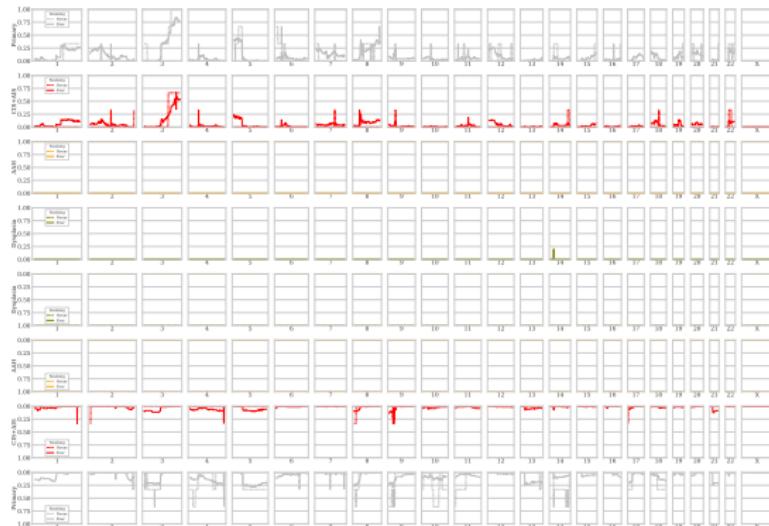


Figure: LUSC with Smoking in CNV Plot

LUSC with Smoking in CNV Plot II

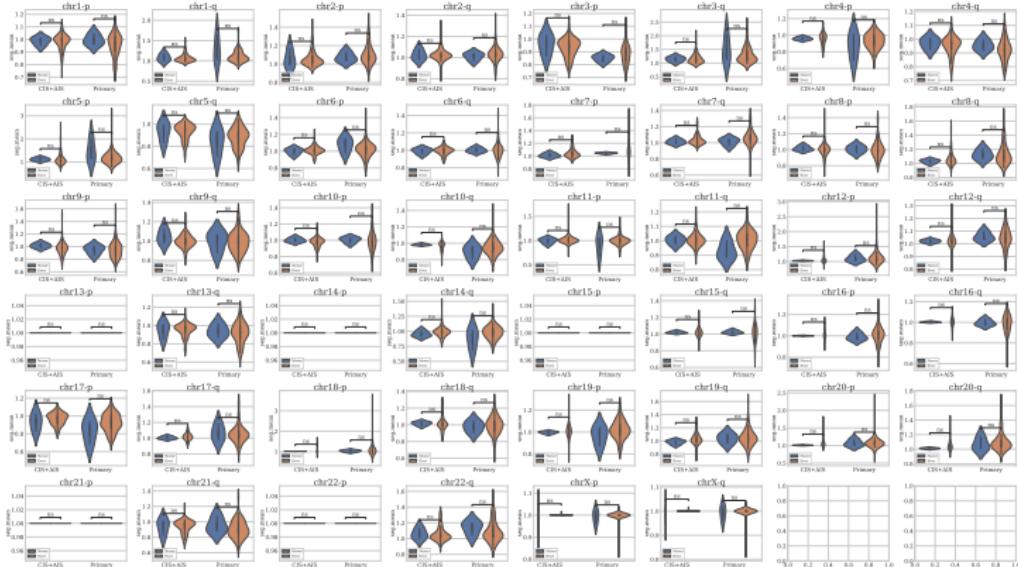


Figure: LUSC with Smoking in Violin Plots

Findings in PureCN with LUSC I

Chr2 p-arm gain

- ① Chr2 p-arm gain in Primary.

Table: CGC Tier1 genes in Chr2 p-arm

| Gene Symbol (15) | Name |
|------------------|--|
| ALK | anaplastic lymphoma kinase (Ki-1) |
| BCL11A | B-cell CLL/lymphoma 11A |
| DCTN1 | dynactin 1 |
| DNMT3A | DNA (cytosine-5-)-methyltransferase 3 alpha |
| EML4 | echinoderm microtubule associated protein like 4 |

Findings in PureCN with LUSC II

Chr2 q-arm loss

- ① Chr2 q-arm loss in Recurrence & Primary.

Table: CGC Tier1 genes in Chr2 q-arm

| Gene Symbol (23) | Name |
|------------------|--|
| ACKR3 | atypical chemokine receptor 3 |
| ACSL3 | acyl-CoA synthetase long-chain family member 3 |
| ACVR1 | activin A receptor, type I |
| ACVR2A | activin A receptor type 2A |
| AFF3 | AF4/FMR2 family, member 3 |

Findings in PureCN with LUSC III

Chr3 p-arm loss

- ① Chr3 p-arm loss in Primary.

Table: CGC Tier1 genes in Chr3 p-arm

| Gene Symbol (17) | Name |
|------------------|--|
| BAP1 | BRCA1 associated protein-1 (ubiquitin carboxy-t... |
| CACNA1D | calcium channel, voltage-dependent, L type, alp... |
| CTNNB1 | catenin (cadherin-associated protein), beta 1 |
| FANCD2 | Fanconi anemia, complementation group D2 |
| FHIT | fragile histidine triad gene |

Findings in PureCN with LUSC IV

Chr3 q-arm gain

- ① Chr3 q-arm gain in Primary.

Table: CGC Tier1 genes in Chr3 q-arm

| Gene Symbol (21) | Name |
|------------------|--|
| ATR | ATR serine/threonine kinase |
| BCL6 | B-cell CLL/lymphoma 6 |
| CBLB | Cas-Br-M (murine) ecotropic retroviral transfor... |
| CNBP | CCHC-type zinc finger, nucleic acid binding pro... |
| EIF4A2 | eukaryotic translation initiation factor 4A, is... |

Findings in PureCN with LUSC V

Chr5 q-arm loss

① Chr5 q-arm loss in Primary.

Table: CGC Tier1 genes in Chr5 q-arm

| Gene Symbol (15) | Name |
|------------------|--|
| AFF4 | AF4/FMR2 family, member 4 |
| APC | adenomatous polyposis of the colon gene |
| ARHGAP26 | Rho GTPase activating protein 26 |
| CD74 | CD74 molecule, major histocompatibility complex... |
| EBF1 | early B-cell factor 1 |

Findings in PureCN with LUSC VI

Chr7 p-arm gain

- ① Chr7 p-arm gain in Primary.
- ② Chr7 p-arm gain in Recurrence.

Table: CGC Tier1 genes in Chr7 p-arm

| Gene Symbol (11) | Name |
|------------------|--|
| CARD11 | caspase recruitment domain family, member 11 |
| EGFR | epidermal growth factor receptor (erythroblasti... |
| ETV1 | ets variant gene 1 |
| HNRNPA2B1 | heterogeneous nuclear ribonucleoprotein A2/B1 |
| HOXA11 | homeo box A11 |

Findings in PureCN with LUSC VII

Chr8 q-arm gain

① Chr8 q-arm gain in Primary.

Table: CGC Tier1 genes in Chr8 q-arm

| Gene Symbol (16) | Name |
|------------------|---|
| CHCHD7 | coiled-coil-helix-coiled-coil-helix domain cont... |
| EIF3E | eukaryotic translation initiation factor 3, sub... |
| EXT1 | multiple exostoses type 1 gene |
| HEY1 | hairy/enhancer-of-split related with YRPW motif 1 |
| MYC | v-myc myelocytomatosis viral oncogene homolog (...) |

Findings in PureCN with LUSC VIII

Chr9 q-arm gain

- Chr9 q-arm gain in Recurrence & Primary.

Table: CGC Tier1 genes in Chr9 q-arm

| Gene Symbol (16) | Name |
|------------------|---|
| ABL1 | v-abl Abelson murine leukemia viral oncogene homolog |
| BRD3 | bromodomain containing 3 |
| CNTRL | centriolin |
| FANCC | Fanconi anemia, complementation group C |
| GNAQ | guanine nucleotide binding protein (G protein), alpha Q subunit |

Findings in PureCN with LUSC IX

Chr14 q-arm gain

- ① Chr14 q-arm gain in Recurrence & Primary.

Table: CGC Tier1 genes in Chr14 q-arm

| Gene Symbol (18) | Name |
|------------------|--|
| AKT1 | v-akt murine thymoma viral oncogene homolog 1 |
| BCL11B | B-cell CLL/lymphoma 11B (CTIP2) |
| CCNB1IP1 | cyclin B1 interacting protein 1, E3 ubiquitin p... |
| DICER1 | dicer 1, ribonuclease type III |
| FOXA1 | forkhead box A1 |

Findings in PureCN with LUSC X

Chr19 p-arm gain

- ① Chr19 p-arm gain in Recurrence & Primary.

Table: CGC Tier1 genes in Chr19 p-arm

| Gene Symbol (19) | Name |
|------------------|--|
| BRD4 | bromodomain containing 4 |
| CALR | calreticulin |
| CRTC1 | CREB regulated transcription coactivator 1 |
| DNAJB1 | DnaJ heat shock protein family (Hsp40) member B1 |
| DNM2 | dynamin 2 |

LUAD in CNV Plot I

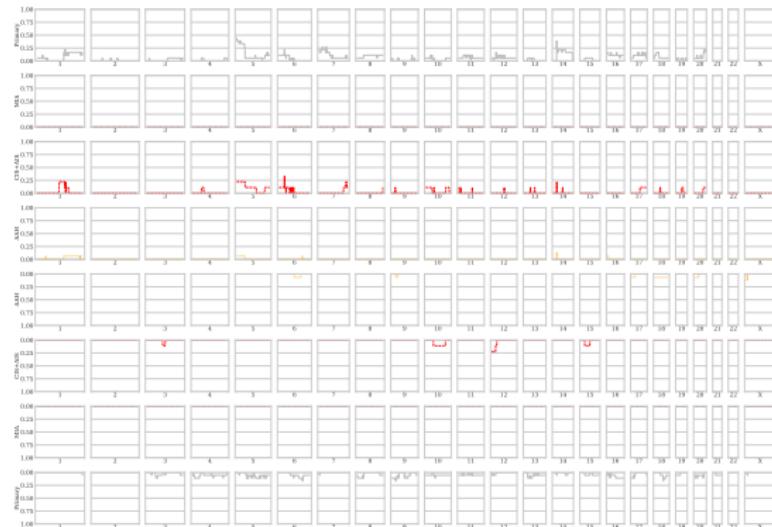


Figure: LUAD in CNV Plot

LUAD in CNV Plot II

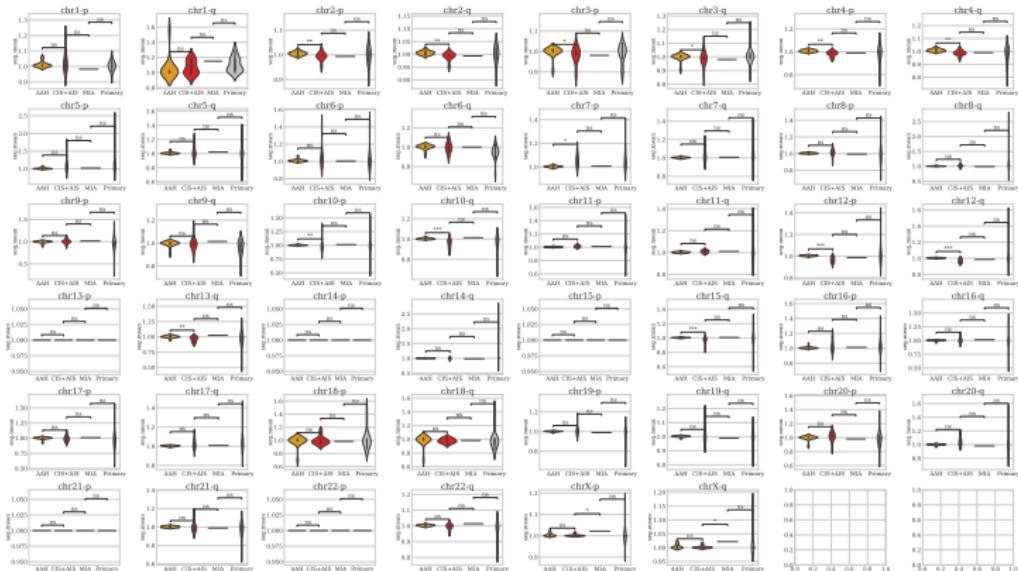


Figure: LUAD in Violin Plots

LUAD with Recurrence in CNV Plot

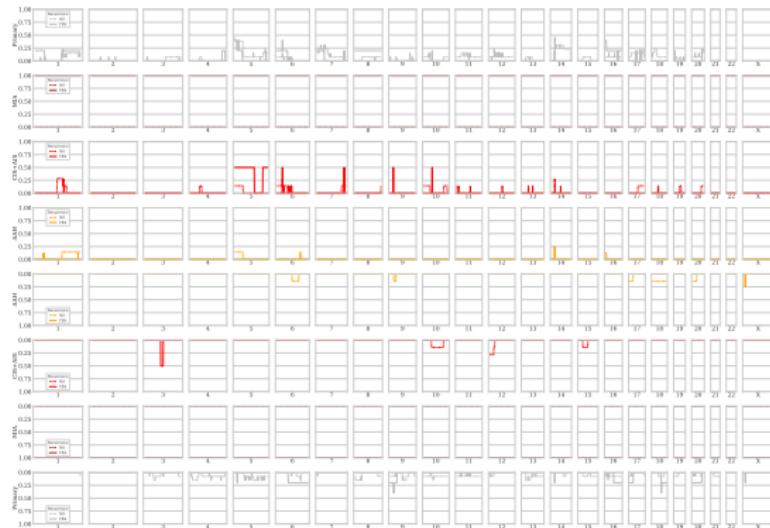


Figure: LUAD with Recurrence in CNV Plot

LUAD with Recurrence in CNV Plot II



Figure: LUAD with Recurrence in Violin Plots

LUAD with Smoking in CNV Plot I



Figure: LUAD with Smoking in CNV Plot

LUAD with Smoking in CNV Plot II

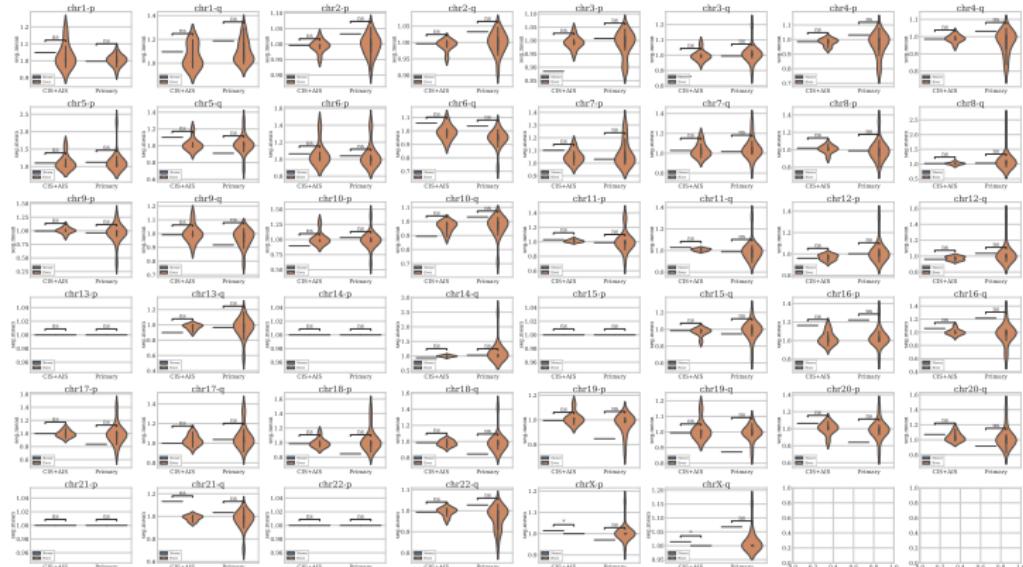


Figure: LUAD with Smoking in Violin Plots

Findings in PureCN with LUAD I

Chr1 q-arm gain

- ① Chr1 q-arm gain in Primary.

Table: CGC Tier1 genes in Chr1 q-arm

| Gene Symbol (24) | Name |
|------------------|--|
| ABL2 | c-abl oncogene 2, non-receptor tyrosine kinase |
| ARNT | aryl hydrocarbon receptor nuclear translocator |
| BCL9 | B-cell CLL/lymphoma 9 |
| CDC73 | cell division cycle 73 |
| DDR2 | discoidin domain receptor 2 |

Findings in PureCN with LUAD II

Chr4 q-arm gain

- ① Chr4 q-arm gain in Recurrence & Primary.

Table: CGC Tier1 genes in Chr4 q-arm

| Gene Symbol (13) | Name |
|------------------|--|
| AFF1 | AF4/FMR2 family, member 1 |
| FAT1 | FAT atypical cadherin 1 |
| FAT4 | FAT atypical cadherin 4 |
| FBXW7 | F-box and WD-40 domain protein 7 (archipelago homolog) |
| FIP1L1 | FIP1 like 1 (<i>S. cerevisiae</i>) |

Findings in PureCN with LUAD III

Chr5 q-arm gain

- ① Chr5 q-arm loss in Non-recurrence & Precancer.

Table: CGC Tier1 genes in Chr5 q-arm

| Gene Symbol (15) | Name |
|------------------|--|
| AFF4 | AF4/FMR2 family, member 4 |
| APC | adenomatous polyposis of the colon gene |
| ARHGAP26 | Rho GTPase activating protein 26 |
| CD74 | CD74 molecule, major histocompatibility complex... |
| EBF1 | early B-cell factor 1 |

Findings in PureCN with LUAD IV

Chr6 p-arm gain

- ① Chr6 p-arm gain in Non-recurrence & Precancer.

Table: CGC Tier1 genes in Chr6 p-arm

| Gene Symbol (16) | Name |
|------------------|---|
| CCND3 | cyclin D3 |
| DAXX | death-domain associated protein |
| DEK | DEK oncogene (DNA binding) |
| FANCE | Fanconi anemia, complementation group E |
| HIST1H3B | histone cluster 1, H3b |

Findings in PureCN with LUAD V

Chr6 q-arm gain

- ① Chr6 q-arm gain in Non-recurrence & Precancer.

Table: CGC Tier1 genes in Chr6 q-arm

| Gene Symbol (15) | Name |
|------------------|---|
| AFDN | myeloid/lymphoid or mixed-lineage leukemia (tri...) |
| ARID1B | AT rich interactive domain 1B |
| ESR1 | estrogen receptor 1 |
| EZR | ezrin |
| FGFR1OP | FGFR1 oncogene partner (FOP) |

Findings in PureCN with LUAD VI

Chr6 q-arm loss

- Chr6 q-arm loss in Primary.

Table: CGC Tier1 genes in Chr6 q-arm

| Gene Symbol (15) | Name |
|------------------|---|
| AFDN | myeloid/lymphoid or mixed-lineage leukemia (tri...) |
| ARID1B | AT rich interactive domain 1B |
| ESR1 | estrogen receptor 1 |
| EZR | ezrin |
| FGFR1OP | FGFR1 oncogene partner (FOP) |

Findings in PureCN with LUAD VII

Chr7 q-arm gain

- ① Chr7 q-arm gain in Non-recurrence & Precancer.

Table: CGC Tier1 genes in Chr7 q-arm

| Gene Symbol (14) | Name |
|------------------|--|
| BRAF | v-raf murine sarcoma viral oncogene homolog B1 |
| CDK6 | cyclin-dependent kinase 6 |
| CREB3L2 | cAMP responsive element binding protein 3-like 2 |
| CUX1 | cut-like homeobox 1 |
| EZH2 | enhancer of zeste homolog 2 |

Findings in PureCN with LUAD VIII

Chr12 p-arm gain

- ① Chr12 p-arm loss in Non-recurrence & Primary.

Table: CGC Tier1 genes in Chr12 p-arm

| Gene Symbol (10) | Name |
|------------------|--|
| CCND2 | cyclin D2 |
| CDKN1B | cyclin-dependent kinase inhibitor 1B (p27, Kip1) |
| CHD4 | chromodomain helicase DNA binding protein 4 |
| ERC1 | ELKS/RAB6-interacting/CAST family member 1 |
| ETNK1 | ethanolamine kinase 1 |

Findings in PureCN with LUAD IX

Chr12 q-arm gain

- ① Chr12 q-arm loss in Non-recurrence & Primary.

Table: CGC Tier1 genes in Chr12 q-arm

| Gene Symbol (26) | Name |
|------------------|---|
| ARID2 | AT rich interactive domain 2 |
| ATF1 | activating transcription factor 1 |
| BCL7A | B-cell CLL/lymphoma 7A |
| BTG1 | B-cell translocation gene 1, anti-proliferative |
| CDK4 | cyclin-dependent kinase 4 |

Findings in PureCN with LUAD X

Chr22 q-arm loss

- ① Chr22 q-arm loss in Primary.

Table: CGC Tier1 genes in Chr22 q-arm

| Gene Symbol (15) | Name |
|------------------|--|
| APOBEC3B | apolipoprotein B mRNA editing enzyme catalytic ... |
| BCR | breakpoint cluster region |
| CHEK2 | CHK2 checkpoint homolog (S. pombe) |
| CLTCL1 | clathrin, heavy polypeptide-like 1 |
| EP300 | 300 kd E1A-Binding protein gene |

Findings in PureCN

Gistic?

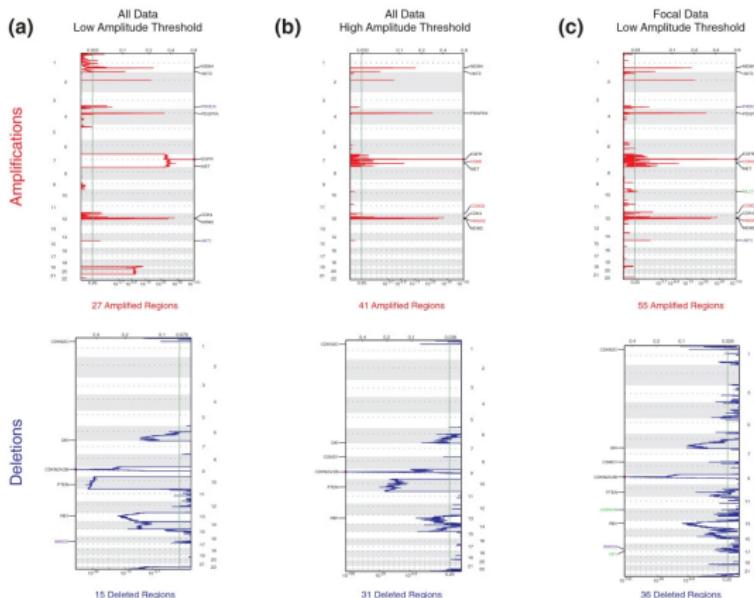


Figure: Effects of arm-level events on GISTIC results (Mermel et al., 2011)

4. Results

4.3. Copy Number Variations

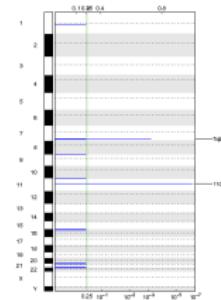
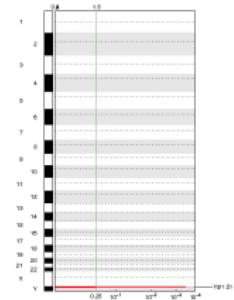
4.3.1. Gistic in LUSC

LUSC Data Composition

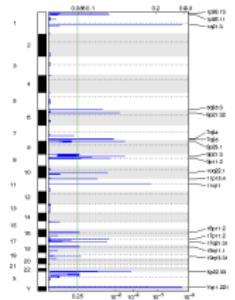
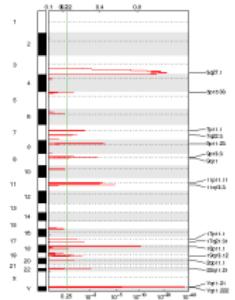
Table: Number of WES samples

| 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 |

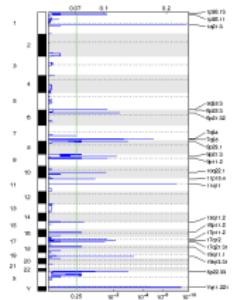
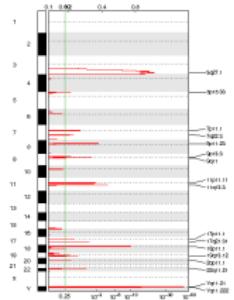
Gistic in LUSC



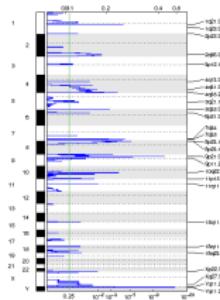
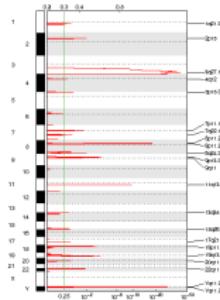
(a) Dysplasia



(b) CIS



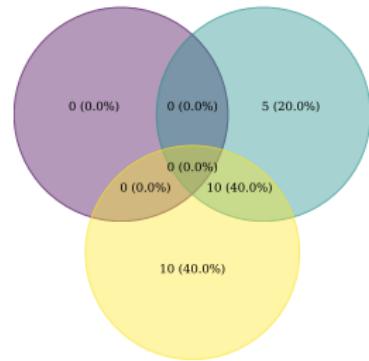
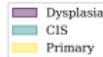
(c) Precancer



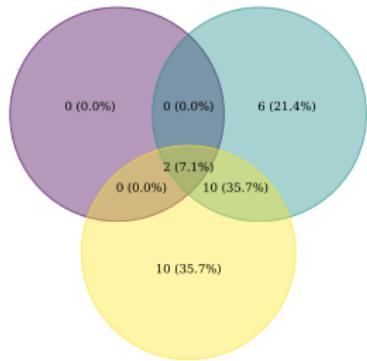
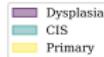
(d) Primary

Figure: Gistic results in LUSC

Venn Diagram in LUSC I



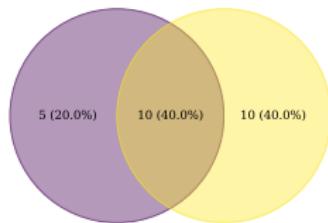
(a) Amplification



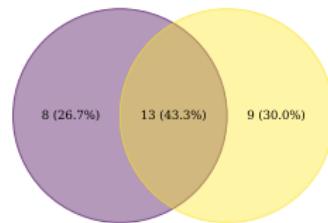
(b) Deletion

Figure: Venn Diagram among Peaks in LUSC – stage

Venn Diagram in LUSC II



(a) Amplification



(b) Deletion

Figure: Venn Diagram among Peaks in LUSC – PRE vs. PRI

Peaks in LUSC I

Table: Peaks in LUSC – stage

(a) Amplification

| | Dysplasia | CIS | Primary | Gene |
|----------|-----------|-----|---------|---------------|
| 1q21.3 | | * | | ARNT,...(3) |
| 2p15 | | * | | XPO1 |
| 3q27.1 | * | * | | |
| 4q12 | | * | | FIP1L1,...(4) |
| 5p15.33 | * | * | | SDHA,TERT |
| 7p11.1 | * | * | | |
| 7q22.1 | | * | | CUX1,TRRAP |
| 7q22.3 | * | | | |
| 8p11.21 | | * | | HOOK3,...(3) |
| 8p11.23 | * | * | | FGFR1,NSD3 |
| 8q24.3 | | * | | RECQL4 |
| 9p13.3 | * | * | | FANCG |
| 9q11 | * | * | | |
| 11p11.11 | * | | | |
| 11q13.3 | * | * | | CCND1 |
| 13q34 | | * | | |
| 15q26.3 | | * | | |
| 17p11.1 | * | | | |
| 17q21.31 | * | * | | BRCA1,ETV4 |
| 18p11.1 | * | * | | |
| 19q13.2 | | * | | AKT2,...(3) |
| 19q13.12 | * | | | |
| 20p11.1 | * | | | |
| 20q11.22 | * | | | |
| 22q11.21 | * | * | | CLTCL1,LZTR1 |

(b) Deletion

| | Dysplasia | CIS | Primary | Gene |
|----------|-----------|-----|---------|--------------|
| 1p36.11 | | * | | ARID1A |
| 1p36.13 | | * | | PAX7,...(3) |
| 1q21.3 | | * | * | ARNT,...(3) |
| 1q25.3 | | | | |
| 2p25.3 | | | | * |
| 2q36.3 | | | | * |
| 3p12.1 | | | | * |
| 4q13.3 | | | | * |
| 4q31.3 | | | | FBXW7 |
| 4q35.2 | | | | FAT1 |
| 5q21.1 | | | | * |
| 5q35.3 | | * | * | FLT4,NSD1 |
| 6p21.32 | | * | * | DAXX |
| 7q34 | * | * | * | BRAF,TRIM24 |
| 7q35 | | * | * | |
| 8p23.1 | | * | * | |
| 9p11.2 | | * | * | |
| 9p21.3 | | * | * | CDKN2A,MLLT3 |
| 10q22.1 | | * | * | PRF1 |
| 11p15.4 | | * | * | CARS,...(3) |
| 11q11 | * | * | * | |
| 15q11.2 | | | | * |
| 16p11.2 | | | | FUS |
| 17p11.2 | | | | |
| 17q21.31 | | | | FLCN,NCOR1 |
| 18q11.1 | | * | * | BRCA1,ETV4 |
| 18q23 | | | | |
| 19q13.31 | | * | | |

Peaks in LUSC II

Table: Peaks in LUSC – PRE vs. PRI

(a) Amplification

| Precancer | Primary | Gene |
|-----------|---------|---------------|
| 1q21.3 | * | ARNT,...(3) |
| 2p15 | * | XPO1 |
| 3q27.1 | * | |
| 4q12 | * | FIP1L1,...(4) |
| 5p15.33 | * | SDHA,TERT |
| 7p11.1 | * | |
| 7q22.1 | * | CUX1,TRRAP |
| 7q22.3 | * | |
| 8p11.21 | * | HOOK3,...(3) |
| 8p11.23 | * | FGFR1,NSD3 |
| 8q24.3 | * | RECQL4 |
| 9p13.3 | * | FANCG |
| 9q11 | * | |
| 11p11.11 | * | |
| 11q13.3 | * | CCND1 |
| 13q34 | * | |
| 15q26.3 | * | |
| 17p11.1 | * | |
| 17q21.31 | * | BRCA1,ETV4 |
| 18p11.1 | * | |
| 19q13.2 | * | AKT2,...(3) |
| 19q13.12 | * | |
| 20p11.1 | * | |
| 20q11.22 | * | |
| 22q11.21 | * | CLTC1,LZTR1 |

(b) Deletion

| Precancer | Primary | Gene |
|-----------|---------|--------------|
| 1p36.11 | * | |
| 1p36.13 | * | |
| 1q21.3 | * | ARID1A |
| 1q25.3 | * | PAX7,...(3) |
| 2p25.3 | * | ARNT,...(3) |
| 2q36.3 | * | |
| 3p12.1 | * | |
| 4q13.3 | * | |
| 4q31.3 | * | FBXW7 |
| 4q35.2 | * | FAT1 |
| 5q21.1 | * | |
| 5q35.3 | * | |
| 6p21.32 | * | FLT4,NSD1 |
| 6p25.3 | * | DAXX |
| 7q34 | * | IRF4 |
| 7q35 | * | BRAF,TRIM24 |
| 8p23.1 | * | |
| 9p11.2 | * | |
| 9p21.3 | * | CDKN2A,MLLT3 |
| 10q22.1 | * | PRF1 |
| 11p15.4 | * | CARS,...(3) |
| 11q11 | * | |
| 15q11.2 | * | |
| 16p11.2 | * | FUS |
| 17p11.2 | * | FLCN,NCOR1 |
| 17q12 | * | CDK12,...(5) |
| 17q21.31 | * | BRCA1,ETV4 |
| 18q11.1 | * | |
| 18q23 | * | |
| 19q13.31 | * | |

4. Results

4.3. Copy Number Variations

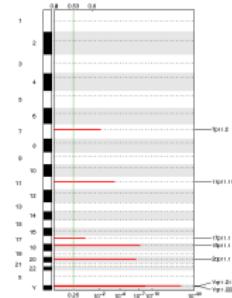
4.3.2. Gistic in LUAD

LUAD Data Composition

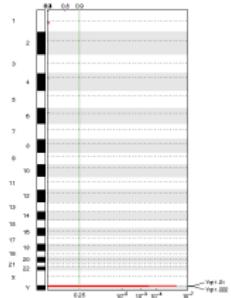
Table: Number of WES samples

| 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 |

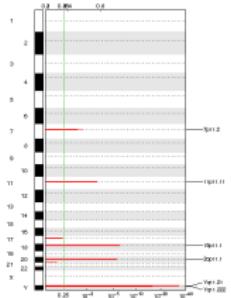
Gistic in LUAD



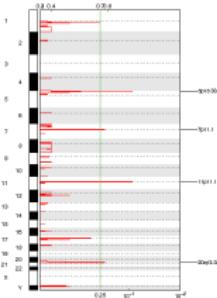
(a) AAH



(b) AIS



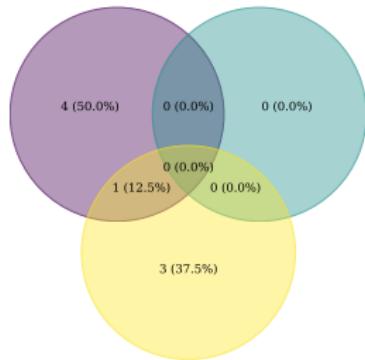
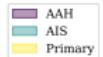
(c) Precancer



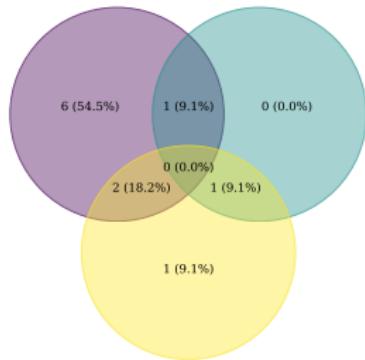
(d) Primary

Figure: Gistic results in LUAD

Venn Diagram in LUAD I



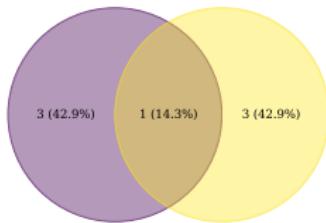
(a) Amplification



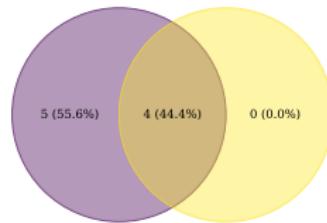
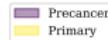
(b) Deletion

Figure: Venn Diagram among Peaks in LUAD – stage

Venn Diagram in LUAD II



(a) Amplification



(b) Deletion

Figure: Venn Diagram among Peaks in LUAD – PRE vs. PRI

Peaks in LUAD I

Table: Peaks in LUAD – stage

| (a) Amplification | | | (b) Deletion | | | | |
|-------------------|-----|---------|--------------|---------|-----|---------|-----------------|
| AAH | AIS | Primary | Gene | AAH | AIS | Primary | Gene |
| 5p15.33 | | * | SDHA,TERT | 1q21.3 | * | * | ARNT,...(3) |
| 7p11.1 | | * | | 1q44 | | * | |
| 7p11.2 | * | | EGFR | 5q35.3 | * | | FLT4,NSD1 |
| 11p11.11 | * | * | | 7q35 | * | * | |
| 17p11.1 | * | | | 9p11.2 | * | | |
| 18p11.1 | * | | | 9p21.3 | * | | CDKN2A,MLLT3 |
| 20p11.1 | * | | | 9q13 | * | * | |
| 20q13.33 | | * | PTK6,SS18L1 | 10q22.1 | * | | PRF1 |
| | | | | 11q11 | * | * | |
| | | | | 12p13.2 | * | | ETV6 |
| | | | | 22q13.1 | * | | APOBEC3B,...(3) |

Peaks in LUAD II

Table: Peaks in LUAD – PRE vs. PRI

| (a) Amplification | | | (b) Deletion | | |
|-------------------|---------|-------------|--------------|---------|-----------------|
| Precancer | Primary | Gene | Precancer | Primary | Gene |
| 5p15.33 | * | SDHA,TERT | 1q21.3 | * | ARNT,...(3) |
| 7p11.1 | * | | 1q44 | * | * |
| 7p11.2 | * | | 7q35 | * | * |
| 11p11.11 | * | * | 9p11.2 | * | |
| 18p11.1 | * | | 9q13 | * | * |
| 20p11.1 | * | | 10q22.1 | * | PRF1 |
| 20q13.33 | * | PTK6,SS18L1 | 11q11 | * | * |
| | | | 17q21.31 | * | BRCA1,ETV4 |
| | | | 22q13.1 | * | APOBEC3B,...(3) |

4. Results

4.3. Copy Number Variations

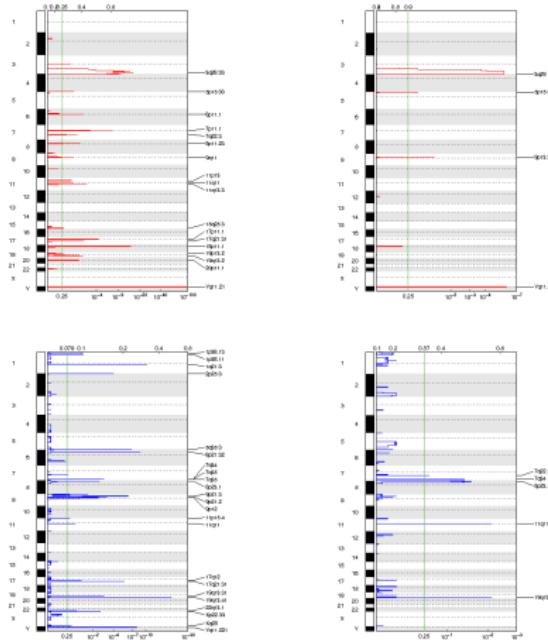
4.3.3. Gistic in Recurrence & LUSC

LUSC Data Composition 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 | |

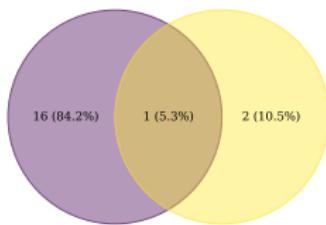
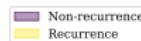
Gistic in Recurrence & LUSC – CIS



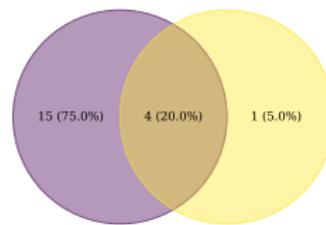
(a) Non-recurrence (b) Recurrence

Figure: Gistic results in Recurrence & LUSC – CIS

Venn Diagram in Recurrence & LUSC – CIS



(a) Amplification



(b) Deletion

Figure: Venn Diagram among Peaks in Recurrence & LUSC – CIS

Peaks in Recurrence & LUSC – CIS

Table: Peaks in Recurrence & LUSC – CIS

| (a) Amplification | | | (b) Deletion | | |
|-------------------|----------------|------------|--------------|----------------|------------|
| | Non-recurrence | Recurrence | | Non-recurrence | Recurrence |
| 3q26.33 | * | | SOX2 | 1p36.11 | * |
| 3q29 | | * | | 1p36.13 | * |
| 5p15.33 | * | * | SDHA,TERT | 1q21.3 | * |
| 6p11.1 | * | | | 2p25.3 | * |
| 7p11.1 | * | | | 5q35.3 | * |
| 7q22.3 | * | | | 6p21.32 | * |
| 8p11.23 | * | | FGFR1,NSD3 | 7q22.1 | * |
| 9p13.3 | | * | FANCG | 7q34 | * |
| 9q11 | * | | | 7q35 | * |
| 11p13 | * | | LMO2,WT1 | 8p23.1 | * |
| 11q11 | * | | | 9p12 | * |
| 11q13.3 | * | | CCND1 | 9p21.2 | * |
| 15q25.3 | * | | NTRK3 | 9p21.3 | |
| 17p11.1 | * | | | 11p15.4 | * |
| 17q21.31 | * | | BRCA1,ETV4 | 11q11 | * |
| 18p11.1 | * | | | 17q12 | * |
| 19p13.2 | * | | DNM2,...(3) | 17q21.31 | * |
| 19q13.2 | * | | AKT2,...(3) | 19q13.31 | * |
| 20p11.1 | * | | | 19q13.41 | * |
| | | | | 22q13.1 | * |

Gistic in Recurrence & LUSC – Precancer

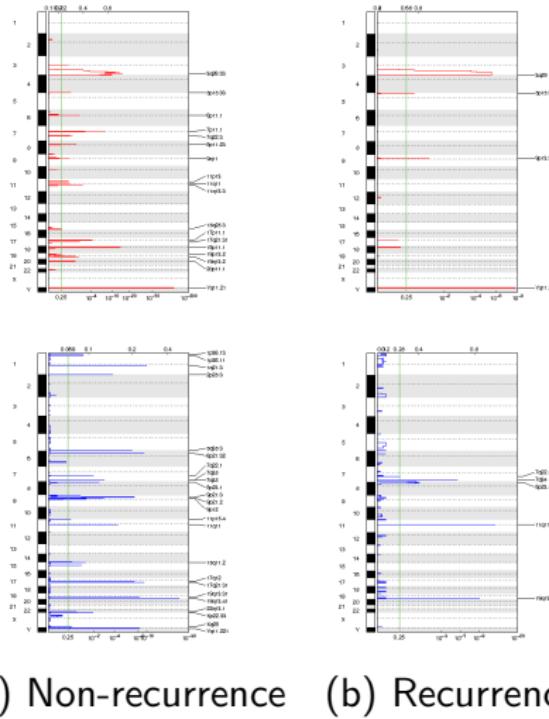
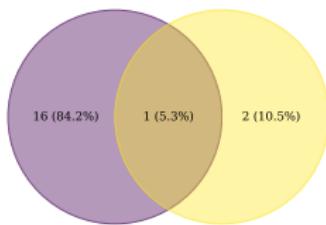
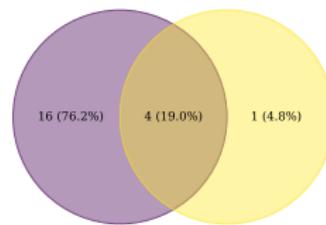


Figure: Gistic results in Recurrence & LUSC – Precancer

Venn Diagram in Recurrence & LUSC – Precancer



(a) Amplification



(b) Deletion

Figure: Venn Diagram among Peaks in Recurrence & LUSC – Precancer

Peaks in Recurrence & LUSC – Precancer

Table: Peaks in Recurrence & LUSC – Precancer

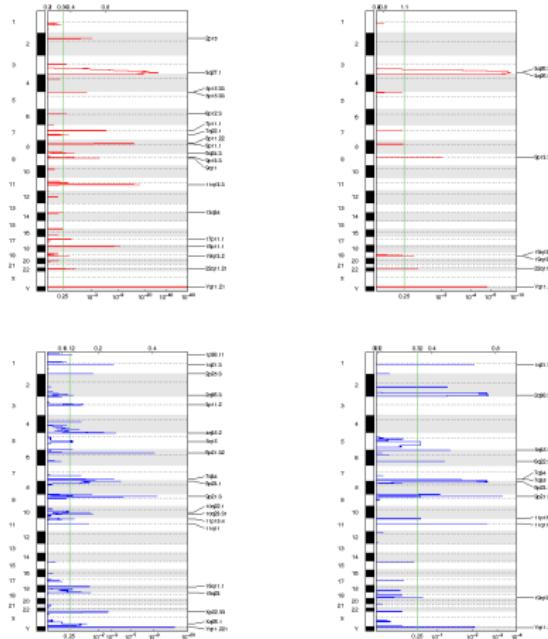
(a) Amplification

| | Non-recurrence | Recurrence | Gene |
|----------|----------------|------------|-------------|
| 3q26.33 | * | | SOX2 |
| 3q29 | | * | |
| 5p15.33 | * | * | SDHA,TERT |
| 6p11.1 | * | | |
| 7p11.1 | * | | |
| 7q22.3 | * | | |
| 8p11.23 | * | | FGFR1,NSD3 |
| 9p13.3 | | * | FANCG |
| 9q11 | * | | |
| 11p13 | * | | LMO2,WT1 |
| 11q11 | * | | |
| 11q13.3 | * | | CCND1 |
| 15q25.3 | * | | NTRK3 |
| 17p11.1 | * | | |
| 17q21.31 | * | | BRCA1,ETV4 |
| 18p11.1 | * | | |
| 19p13.2 | * | | DNM2,...(3) |
| 19q13.2 | * | | AKT2,...(3) |
| 20p11.1 | * | | |

(b) Deletion

| | Non-recurrence | Recurrence | Gene |
|----------|----------------|------------|-----------------|
| 1p36.11 | * | | ARID1A |
| 1p36.13 | * | | PAX7,...(3) |
| 1q21.3 | * | | ARNT,...(3) |
| 2p25.3 | * | | |
| 5q35.3 | * | | FLT4,NSD1 |
| 6p21.32 | * | | DAXX |
| 7q22.1 | * | * | CUX1,TRRAP |
| 7q34 | | * | BRAF,TRIM24 |
| 7q35 | * | | |
| 8p23.1 | * | * | |
| 9p12 | * | | |
| 9p21.2 | * | | |
| 9p21.3 | * | | CDKN2A,MLLT3 |
| 11p15.4 | * | | CARS,...(3) |
| 11q11 | * | * | |
| 15q11.2 | * | | |
| 17q12 | * | | CDK12,...(5) |
| 17q21.31 | * | | BRCA1,ETV4 |
| 19q13.31 | * | | |
| 19q13.41 | * | * | PPP2R1A |
| 22q13.1 | * | | APOBEC3B,...(3) |

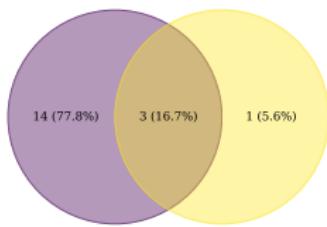
Gistic in Recurrence & LUSC – Primary



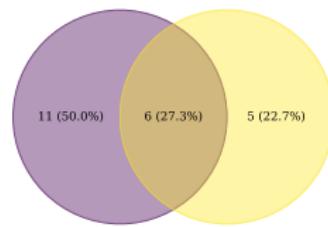
(a) Non-recurrence (b) Recurrence

Figure: Gistic results in Recurrence & LUSC – Primary

Venn Diagram in Recurrence & LUSC – Primary



(a) Amplification



(b) Deletion

Figure: Venn Diagram among Peaks in Recurrence & LUSC – Primary

Peaks in Recurrence & LUSC – Primary

Table: Peaks in Recurrence & LUSC – Primary

(a) Amplification

| | Non-recurrence | Recurrence | Gene |
|----------|----------------|------------|-------------|
| 2p15 | * | | XPO1 |
| 3q26.33 | | * | SOX2 |
| 3q27.1 | * | | |
| 5p15.33 | * | | SDHA,TERT |
| 6p12.3 | * | | |
| 7p11.1 | * | | |
| 7q22.1 | * | | CUX1,TRRAP |
| 8p11.1 | * | | |
| 8p11.22 | * | | |
| 8q24.3 | * | | RECQL4 |
| 9p13.3 | * | * | FANCG |
| 9q11 | * | | |
| 11q13.3 | * | | CCND1 |
| 13q34 | * | | |
| 17p11.1 | * | | |
| 18p11.1 | * | | |
| 19q13.2 | * | * | AKT2,...(3) |
| 22q11.21 | * | * | CLTC1,LZTR1 |

(b) Deletion

| | Non-recurrence | Recurrence | Gene |
|----------|----------------|------------|--------------|
| 1p36.11 | * | | ARID1A |
| 1q21.3 | * | * | ARNT,...(3) |
| 2p25.3 | * | | |
| 2q36.3 | * | * | |
| 3p11.2 | * | | |
| 4q35.2 | * | | FAT1 |
| 5q15 | * | | |
| 5q35.3 | | * | FLT4,NSD1 |
| 6p21.32 | * | | DAXX |
| 6q22.31 | | * | |
| 7q34 | * | * | BRAF,TRIM24 |
| 7q35 | | * | |
| 8p23.1 | * | * | |
| 9p21.3 | * | * | CDKN2A,MLLT3 |
| 10q22.1 | * | | PRF1 |
| 10q23.31 | * | | FAS,PTEN |
| 11p15.4 | * | | CARS,...(3) |
| 11p15.5 | | * | HRAS |
| 11q11 | * | * | |
| 18q11.1 | * | | |
| 18q23 | * | | |
| 19q13.43 | | * | |

4. Results

4.3. Copy Number Variations

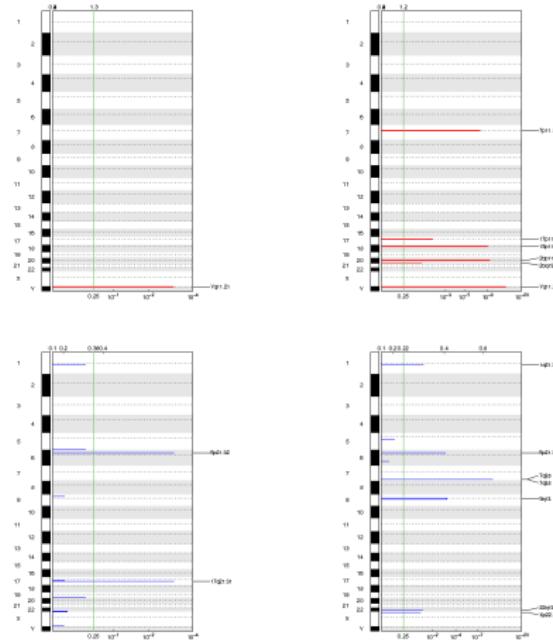
4.3.4. Gistic in Recurrence & LUAD

LUAD Data Composition with Recurrence

Table: LUAD WES Data with Recurrence

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

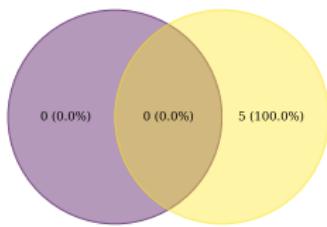
Gistic in Recurrence & LUAD – AAH



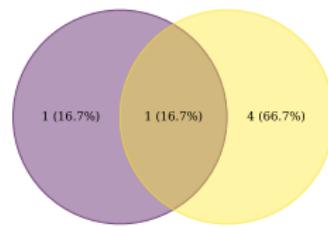
(a) Non-recurrence (b) Recurrence

Figure: Gistic results in Recurrence & LUAD – AAH

Venn Diagram in Recurrence & LUAD – AAH



(a) Amplification



(b) Deletion

Figure: Venn Diagram among Peaks in Recurrence & LUAD – AAH

Peaks in Recurrence & LUAD – AAH

Table: Peaks in Recurrence & LUAD – AAH

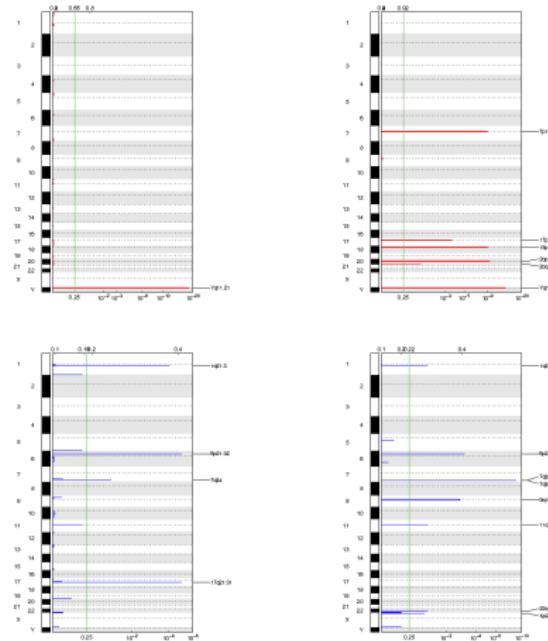
(a) Amplification

| Non-recurrence | Recurrence | Gene |
|----------------|------------|-------------|
| 7p11.1 | * | |
| 17p11.1 | * | |
| 18p11.1 | * | |
| 20p11.1 | * | |
| 20q13.33 | * | PTK6,SS18L1 |

(b) Deletion

| Non-recurrence | Recurrence | Gene |
|----------------|------------|-----------------|
| 1q21.3 | * | ARNT,...(3) |
| 6p21.32 | * | DAXX |
| 7q35 | | * |
| 9q13 | | * |
| 17q21.31 | * | BRCA1,ETV4 |
| 22q13.1 | * | APOBEC3B,...(3) |

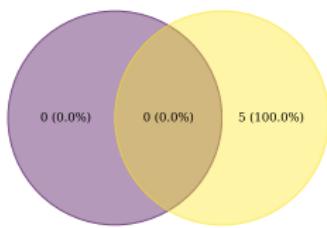
Gistic in Recurrence & LUAD – Precancer



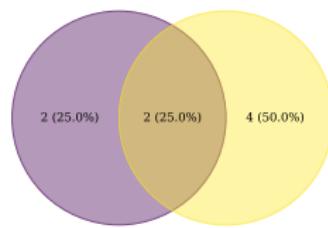
(a) Non-recurrence (b) Recurrence

Figure: Gistic results in Recurrence & LUAD – Precancer

Venn Diagram in Recurrence & LUAD – Precancer



(a) Amplification



(b) Deletion

Figure: Venn Diagram among Peaks in Recurrence & LUAD – Precancer

Peaks in Recurrence & LUAD – Precancer

Table: Peaks in Recurrence & LUAD – Precancer

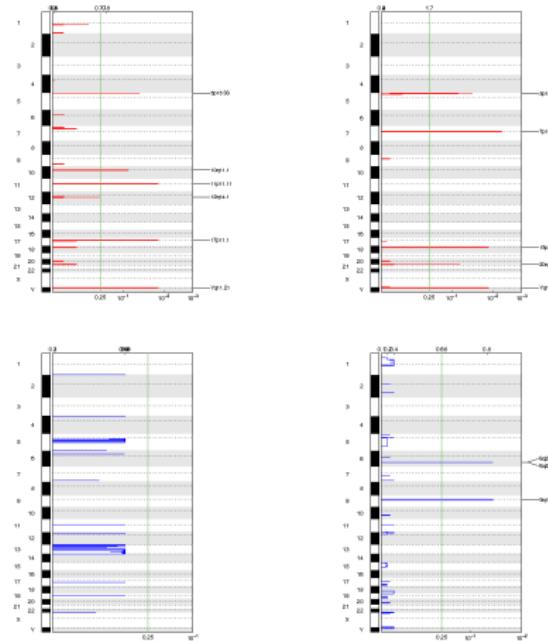
(a) Amplification

| Non-recurrence | Recurrence | Gene |
|----------------|------------|-------------|
| 7p11.1 | * | |
| 17p11.1 | * | |
| 18p11.1 | * | |
| 20p11.1 | * | |
| 20q13.33 | * | PTK6,SS18L1 |

(b) Deletion

| Non-recurrence | Recurrence | Gene |
|----------------|------------|-----------------|
| 1q21.3 | * | ARNT,...(3) |
| 6p21.32 | * | DAXX |
| 7q34 | * | BRAF,TRIM24 |
| 7q35 | | * |
| 9q13 | | * |
| 11q11 | | * |
| 17q21.31 | * | BRCA1,ETV4 |
| 22q13.1 | * | APOBEC3B,...(3) |

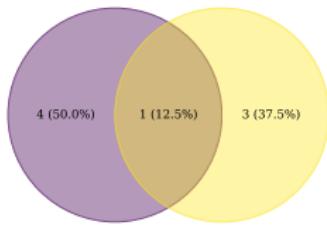
Gistic in Recurrence & LUAD – Primary



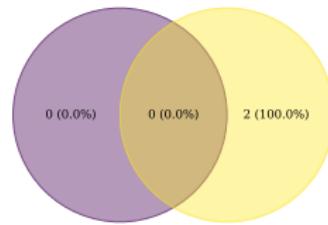
(a) Non-recurrence (b) Recurrence

Figure: Gistic results in Recurrence & LUAD – Primary

Venn Diagram in Recurrence & LUAD – Primary



(a) Amplification



(b) Deletion

Figure: Venn Diagram among Peaks in Recurrence & LUAD – Primary

Peaks in Recurrence & LUAD – Primary

Table: Peaks in Recurrence & LUAD – Primary

(a) Amplification

| | Non-recurrence | Recurrence | Gene |
|----------|----------------|------------|-------------|
| 5p15.33 | * | * | SDHA,TERT |
| 7p11.1 | | * | |
| 10q11.1 | * | | |
| 11p11.11 | * | | |
| 12q14.1 | * | | CDK4,LRIG3 |
| 17p11.1 | * | | |
| 18p11.1 | | * | |
| 20q13.33 | | * | PTK6,SS18L1 |

(b) Deletion

| | Non-recurrence | Recurrence | Gene |
|---------|----------------|------------|------|
| 6q22.31 | | | * |
| 9q13 | | | * |

4. Results

4.3. Copy Number Variations

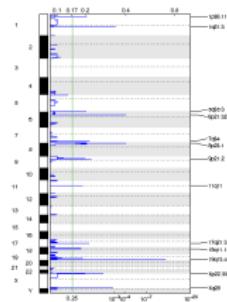
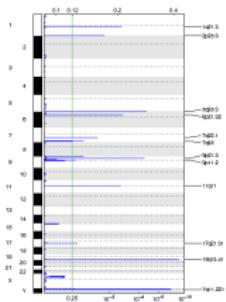
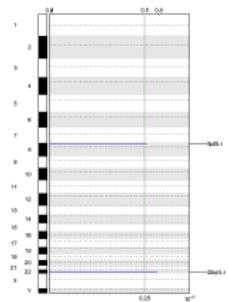
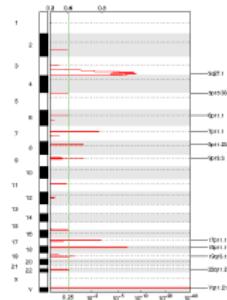
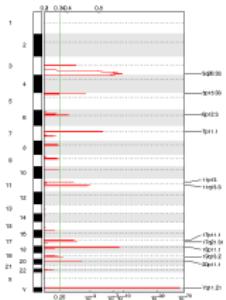
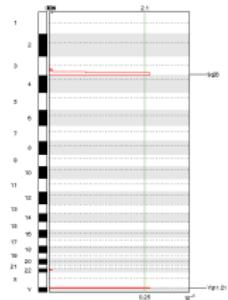
4.3.5. Gistic in Smoking & LUSC

LUSC Data Composition with Smoking

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 | |

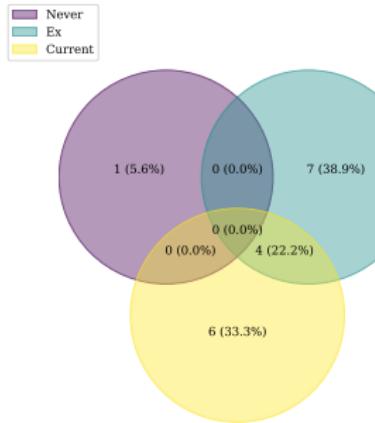
Gistic in Smoking & LUSC – CIS



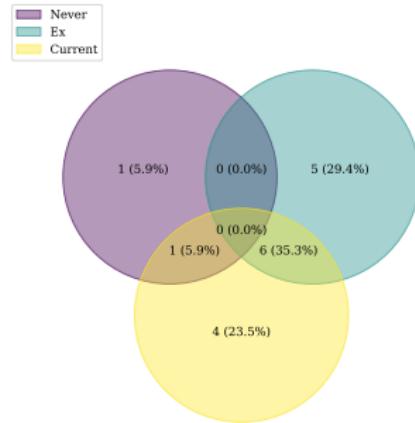
(a) Never Smoker (b) Ex-Smoker (c) Current Smoker

Figure: Gistic results in Smoking & LUSC – CIS

Venn Diagram in Smoking & LUSC – CIS



(a) Amplification



(b) Deletion

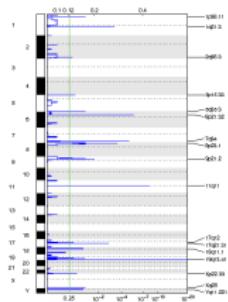
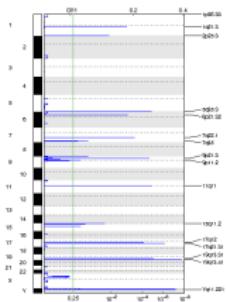
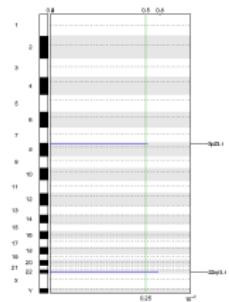
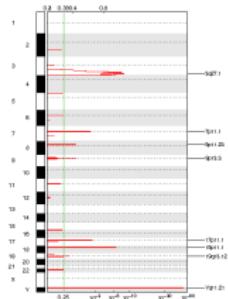
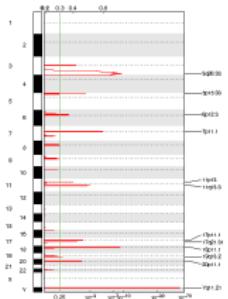
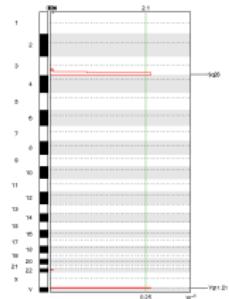
Figure: Venn Diagram among Peaks in Smoking & LUSC – CIS

Peaks in Smoking & LUSC – CIS

Table: Peaks in Smoking & LUSC – CIS

| (a) Amplification | | | | (b) Deletion | | | |
|-------------------|----|---------|--------------|--------------|----|---------|-----------------|
| Never | Ex | Current | Gene | Never | Ex | Current | Gene |
| 3q26.33 | * | | SOX2 | 1p36.11 | | * | ARID1A |
| 3q27.1 | | * | | 1q21.3 | | * | ARNT,...(3) |
| 3q28 | * | | LPP,TP63 | 2p25.3 | | * | |
| 5p15.33 | * | * | SDHA,TERT | 5q35.3 | | * | FLT4,NSD1 |
| 6p11.1 | | * | | 6p21.32 | | * | DAXX |
| 6p12.3 | * | | | 7q22.1 | | * | CUX1,TRRAP |
| 7p11.1 | * | * | | 7q34 | | * | BRAF,TRIM24 |
| 8p11.23 | | * | FGFR1,NSD3 | 7q35 | | * | |
| 9p13.3 | | * | FANCG | 8p23.1 | * | * | |
| 11p13 | * | | LMO2,WT1 | 9p11.2 | | * | |
| 11q13.3 | * | | CCND1 | 9p21.2 | | * | |
| 17p11.1 | * | * | | 9p21.3 | | * | CDKN2A,MLLT3 |
| 17q21.31 | * | | BRCA1,ETV4 | 11q11 | | * | |
| 18p11.1 | * | * | | 17q21.31 | | * | BRCA1,ETV4 |
| 19q13.2 | * | | AKT2,...(3) | 18q11.1 | | * | |
| 19q13.12 | | * | | 19q13.41 | | * | PPP2R1A |
| 20p11.1 | * | | | 22q13.1 | * | | APOBEC3B,...(3) |
| 22q11.21 | | * | CLTCL1,LZTR1 | | | | |

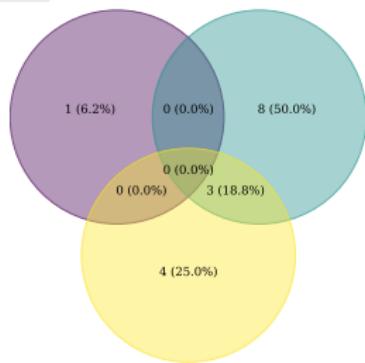
Gistic in Smoking & LUSC – Precancer



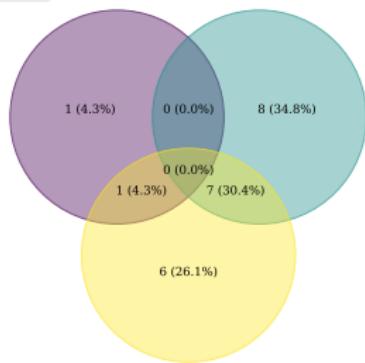
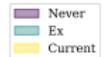
(a) Never Smoker (b) Ex-Smoker (c) Current Smoker

Figure: Gistic results in Smoking & LUSC – Precancer

Venn Diagram in Smoking & LUSC – Precancer



(a) Amplification



(b) Deletion

Figure: Venn Diagram among Peaks in Smoking & LUSC – Precancer

Peaks in Smoking & LUSC – Precancer

Table: Peaks in Smoking & LUSC – Precancer

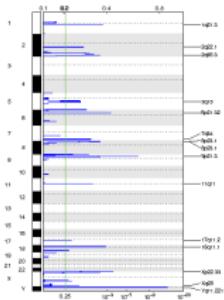
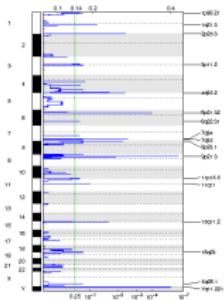
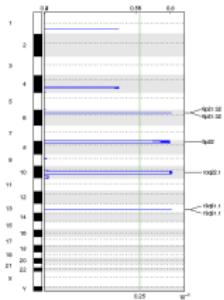
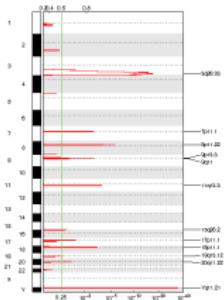
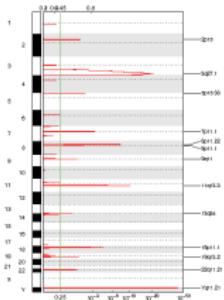
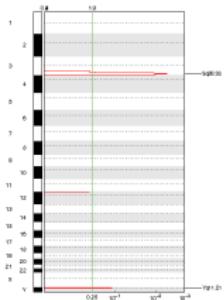
(a) Amplification

| | Never | Ex | Current | Gene |
|----------|-------|----|---------|-------------|
| 3q26.33 | | * | | SOX2 |
| 3q27.1 | | | * | |
| 3q28 | * | | | LPP,TP63 |
| 5p15.33 | | * | | SDHA,TERT |
| 6p12.3 | | * | | |
| 7p11.1 | * | * | | |
| 8p11.23 | | * | | FGFR1,NSD3 |
| 9p13.3 | | * | | FANCG |
| 11p13 | * | | | LMO2,WT1 |
| 11q13.3 | * | | | CCND1 |
| 17p11.1 | * | * | | |
| 17q21.31 | * | | | BRCA1,ETV4 |
| 18p11.1 | * | * | | |
| 19q13.2 | * | | | AKT2,...(3) |
| 19q13.12 | | * | | |
| 20p11.1 | * | | | |

(b) Deletion

| | Never | Ex | Current | Gene |
|----------|-------|----|---------|-----------------|
| 1p36.11 | | | * | |
| 1p36.33 | | | * | ARID1A |
| 1q21.3 | | * | * | |
| 2p25.3 | | * | | ARNT,...(3) |
| 2q36.3 | | | * | |
| 5p15.33 | | | * | SDHA,TERT |
| 5q35.3 | | * | * | FLT4,NSD1 |
| 6p21.32 | | * | * | DAXX |
| 7q22.1 | | * | | CUX1,TRRAP |
| 7q34 | | | * | BRAF,TRIM24 |
| 7q35 | | * | | |
| 8p23.1 | * | | * | |
| 9p11.2 | | * | | |
| 9p21.2 | | | * | |
| 9p21.3 | | * | | CDKN2A,MLLT3 |
| 11q11 | | * | * | |
| 15q11.2 | | * | | |
| 17q12 | | * | * | CDK12,...(5) |
| 17q21.31 | | * | * | BRCA1,ETV4 |
| 18q11.1 | | | * | |
| 19q13.31 | | * | | |
| 19q13.41 | | * | * | PPP2R1A |
| 22q13.1 | * | | | APOBEC3B,...(3) |

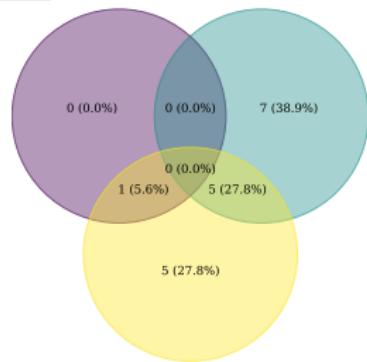
Gistic in Smoking & LUSC – Primary



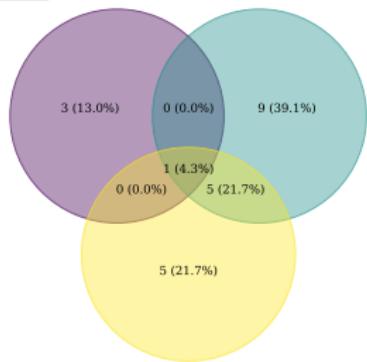
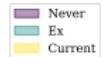
(a) Never Smoker (b) Ex-Smoker (c) Current Smoker

Figure: Gistic results in Smoking & LUSC – Primary

Venn Diagram in Smoking & LUSC – Primary



(a) Amplification



(b) Deletion

Figure: Venn Diagram among Peaks in Smoking & LUSC – Primary

Peaks in Smoking & LUSC – Primary

Table: Peaks in Smoking & LUSC – Primary

(a) Amplification

| | Never | Ex | Current | Gene |
|----------|-------|----|---------|--------------|
| 2p15 | | * | | XPO1 |
| 3q26.33 | * | | * | SOX2 |
| 3q27.1 | * | | | |
| 5p15.33 | * | | | SDHA,TERT |
| 7p11.1 | * | * | | |
| 8p11.1 | * | | | |
| 8p11.22 | * | * | | |
| 9p13.3 | | * | | FANCG |
| 9q11 | * | * | | |
| 11q13.3 | * | * | | CCND1 |
| 13q34 | * | | | |
| 15q26.2 | | * | | |
| 17p11.1 | | * | | |
| 18p11.1 | * | * | | |
| 19q13.2 | * | | | AKT2,...(3) |
| 19q13.12 | | * | | |
| 20q11.22 | | * | | |
| 22q11.21 | * | | | CLTCL1,LZTR1 |

(b) Deletion

| | Never | Ex | Current | Gene |
|---------|-------|----|---------|--------------|
| 1p36.21 | | * | | SPEN |
| 1q21.3 | | * | * | ARNT,...(3) |
| 2p25.3 | | * | | |
| 2q22.1 | | | * | CXCR4,LRP1B |
| 2q36.3 | | | * | |
| 3p11.2 | | * | | |
| 4q35.2 | | * | | FAT1 |
| 5q15 | | | * | |
| 6p21.32 | * | * | * | DAXX |
| 6q22.31 | | * | | |
| 7q34 | | * | * | BRAF,TRIM24 |
| 7q35 | | * | | |
| 8p22 | * | | | PCM1 |
| 8p23.1 | | * | * | |
| 9p21.3 | | * | * | CDKN2A,MLLT3 |
| 10q22.1 | * | | | PRF1 |
| 11p15.5 | | * | | HRAS |
| 11q11 | | * | * | |
| 13q31.1 | * | | | |
| 15q11.2 | | * | | |
| 17q11.2 | | | * | NF1,SUZ12 |
| 18q11.1 | | | * | |
| 18q23 | | * | | |

4. Results

4.3. Copy Number Variations

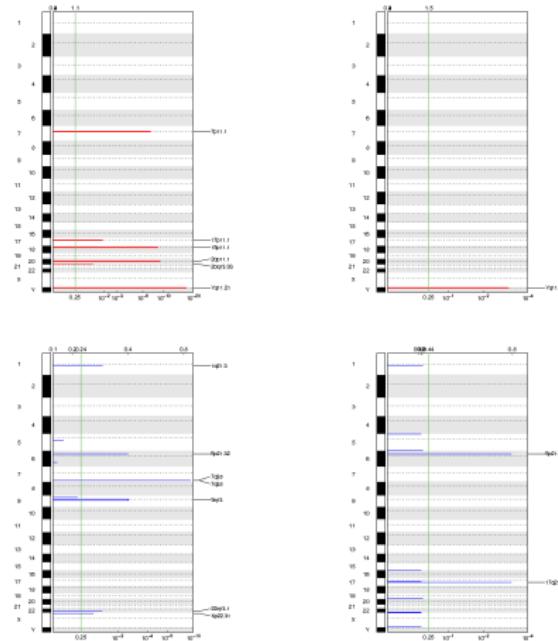
4.3.6. Gistic in Smoking & LUAD

LUAD Data Composition with Smoking

Table: LUAD WES Data with Recurrence

| Smoking? | Stage | Number of Samples | |
|----------|---------|-------------------|----------|
| | | Normal | Affected |
| 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 | |

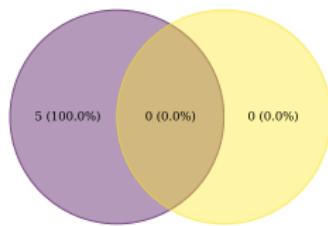
Gistic in Smoking & LUAD – AAH



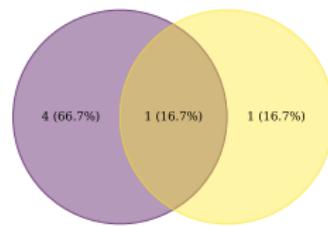
(a) Ex-Smoker (b) Current Smoker

Figure: Gistic results in Smoking & LUAD – AAH

Venn Diagram in Smoking & LUAD – AAH



(a) Amplification



(b) Deletion

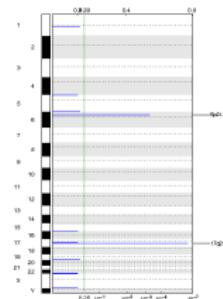
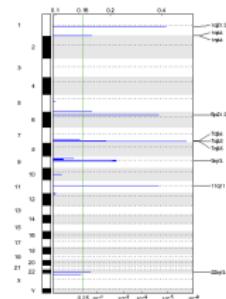
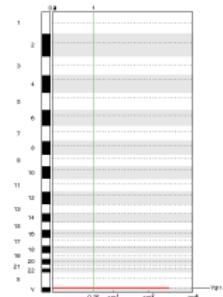
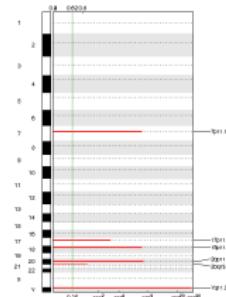
Figure: Venn Diagram among Peaks in Smoking & LUAD – AAH

Peaks in Smoking & LUAD – AAH

Table: Peaks in Smoking & LUAD – AAH

| (a) Amplification | | | (b) Deletion | | |
|-------------------|----|-------------|--------------|----|-----------------|
| | Ex | Current | | Ex | Current |
| 7p11.1 | * | | 1q21.3 | * | ARNT,...(3) |
| 17p11.1 | * | | 6p21.32 | * | DAXX |
| 18p11.1 | * | | 7q35 | * | |
| 20p11.1 | * | | 9q13 | * | |
| 20q13.33 | * | PTK6,SS18L1 | 17q21.31 | * | BRCA1,ETV4 |
| | | | 22q13.1 | * | APOBEC3B,...(3) |

Gistic in Smoking & LUAD – Precancer

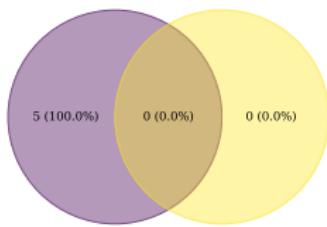


(a) Ex-Smoker

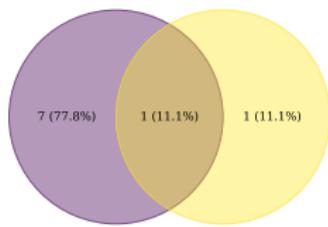
(b) Current Smoker

Figure: Gistic results in Smoking & LUAD – Precancer

Venn Diagram in Smoking & LUAD – Precancer



(a) Amplification



(b) Deletion

Figure: Venn Diagram among Peaks in Smoking & LUAD – Precancer

Peaks in Smoking & LUAD – Precancer

Table: Peaks in Smoking & LUAD – Precancer

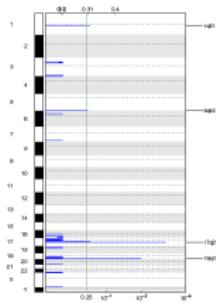
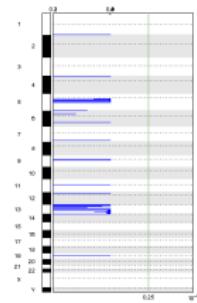
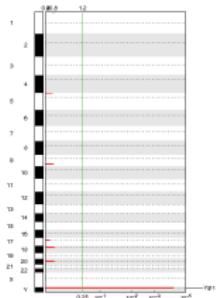
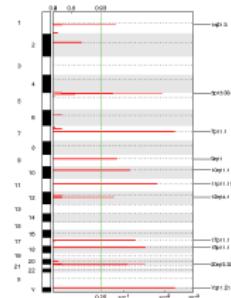
(a) Amplification

| | Ex | Current | Gene |
|----------|----|---------|-------------|
| 7p11.1 | * | | |
| 17p11.1 | * | | |
| 18p11.1 | * | | |
| 20p11.1 | * | | |
| 20q13.33 | * | | PTK6,SS18L1 |

(b) Deletion

| Ex | Current | Gene |
|----------|---------|-----------------|
| 1q21.3 | * | ARNT,...(3) |
| 1q44 | * | |
| 6p21.32 | * | * |
| 7q34 | * | DAXX |
| 7q35 | * | BRAF,TRIM24 |
| 9q13 | * | |
| 11q11 | * | |
| 17q21.31 | | * |
| 22q13.1 | * | APOBEC3B,...(3) |

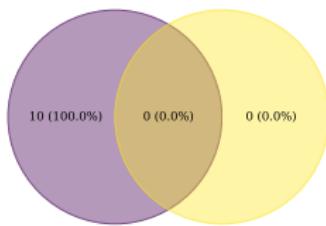
Gistic in Smoking & LUAD – Primary



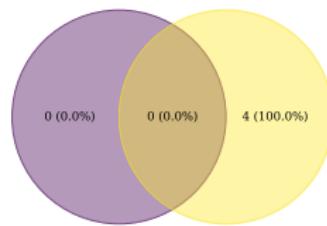
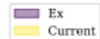
(a) Ex-Smoker (b) Current Smoker

Figure: Gistic results in Smoking & LUAD – Primary

Venn Diagram in Smoking & LUAD – Primary



(a) Amplification



(b) Deletion

Figure: Venn Diagram among Peaks in Smoking & LUAD – Primary

Peaks in Smoking & LUAD – Primary

Table: Peaks in Smoking & LUAD – Primary

(a) Amplification

| | Ex | Current | Gene |
|----------|----|---------|-------------|
| 1q21.3 | * | | ARNT,...(3) |
| 5p15.33 | * | | SDHA,TERT |
| 7p11.1 | * | | |
| 9q11 | * | | |
| 10q11.1 | * | | |
| 11p11.11 | * | | |
| 12q14.1 | * | | CDK4,LRIG3 |
| 17p11.1 | * | | |
| 18p11.1 | * | | |
| 20q13.33 | * | | PTK6,SS18L1 |

(b) Deletion

| | Ex | Current | Gene |
|----------|----|---------|--------------|
| 1q21.3 | * | | ARNT,...(3) |
| 5q35.3 | * | | FLT4,NSD1 |
| 17q21.31 | * | | BRCA1,ETV4 |
| 19q13.42 | * | | CNOT3,ZNF331 |

Findings in Gistic

4. Results

4.4. Single Nucleotide Variations Analysis

Mutect2?

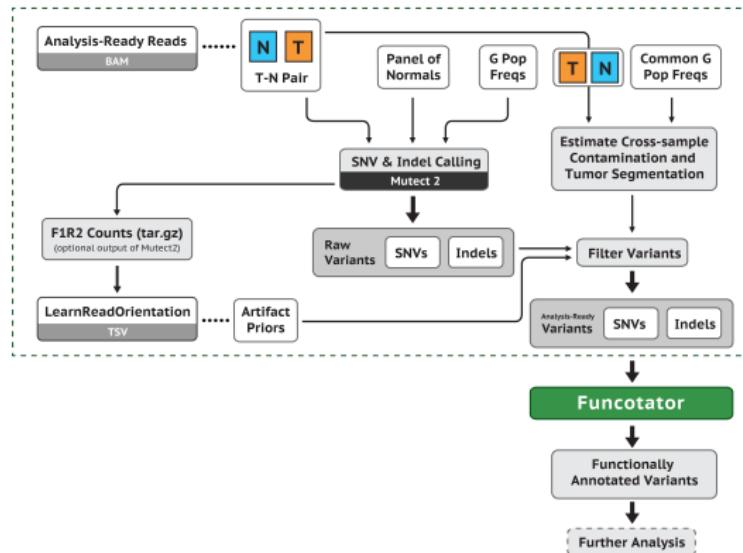
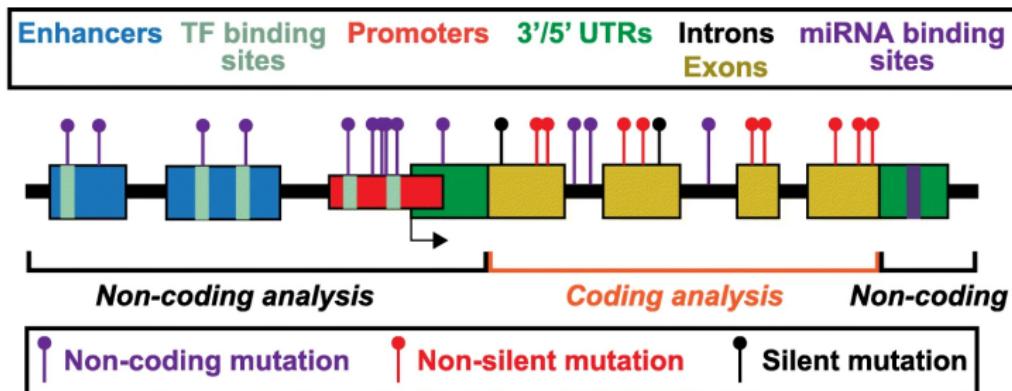


Figure: Somatic short variant discovery workflow (Van der Auwera et al., 2013; DePristo et al., 2011)

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?

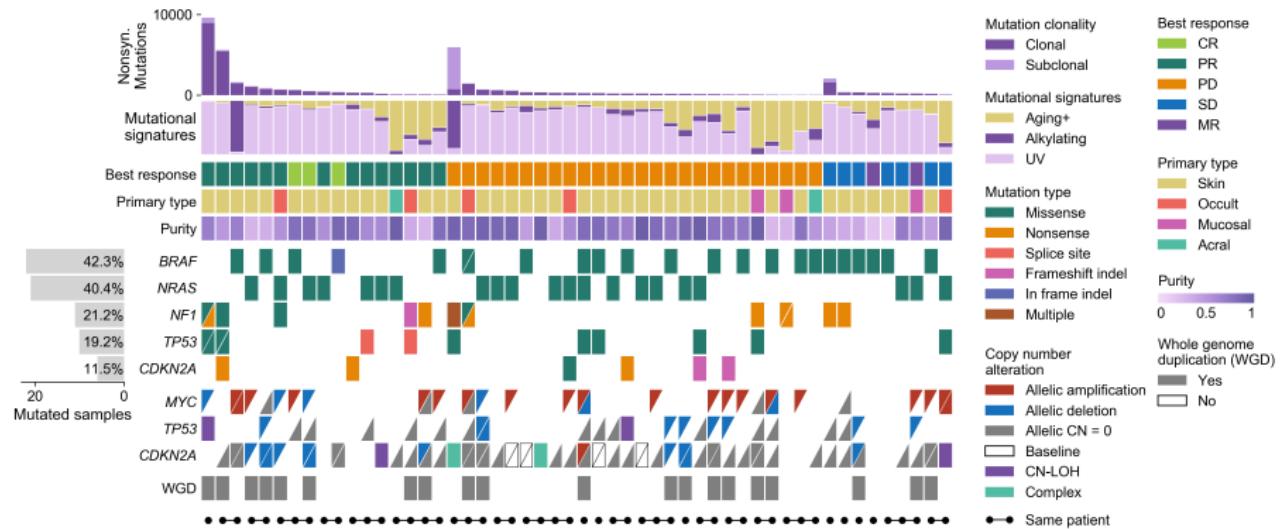


Figure: A comutation plot generated with CoMut (Crowdis et al., 2020)

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

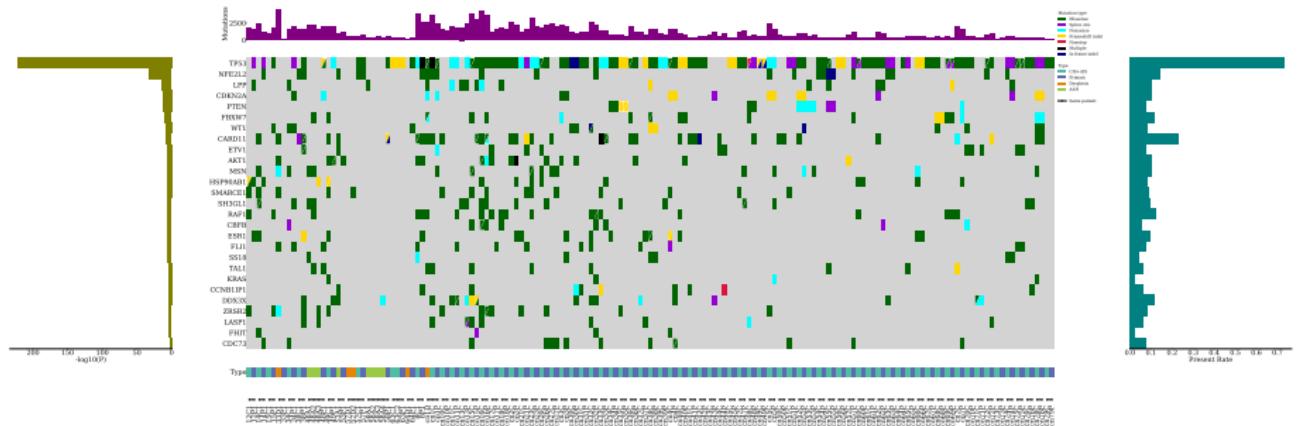


Figure: CoMut Plot with LUSC Patients

Somatic Variant in LUSC with Recurrence

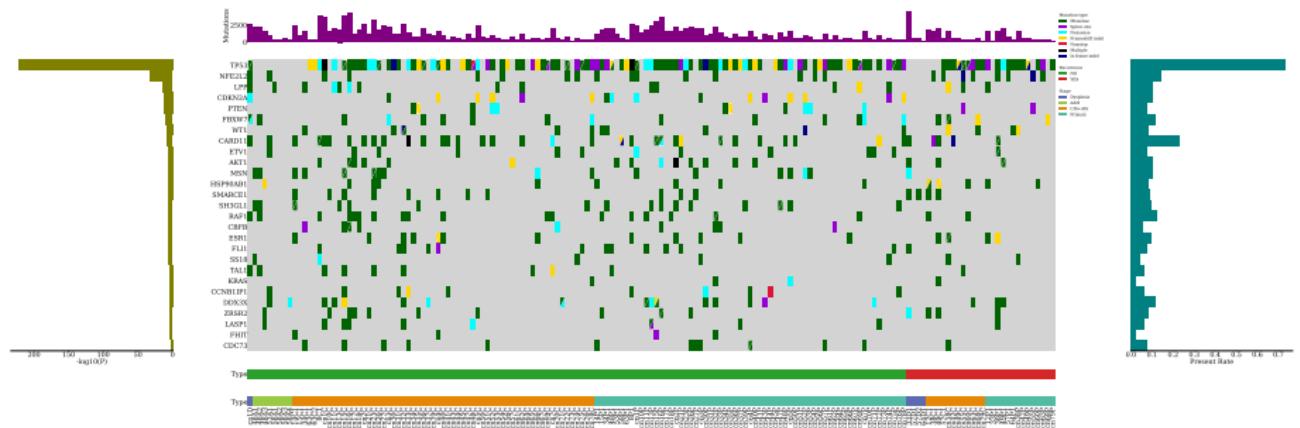


Figure: CoMut Plot in LUSC Patients with Recurrence

Somatic Variant in LUAD

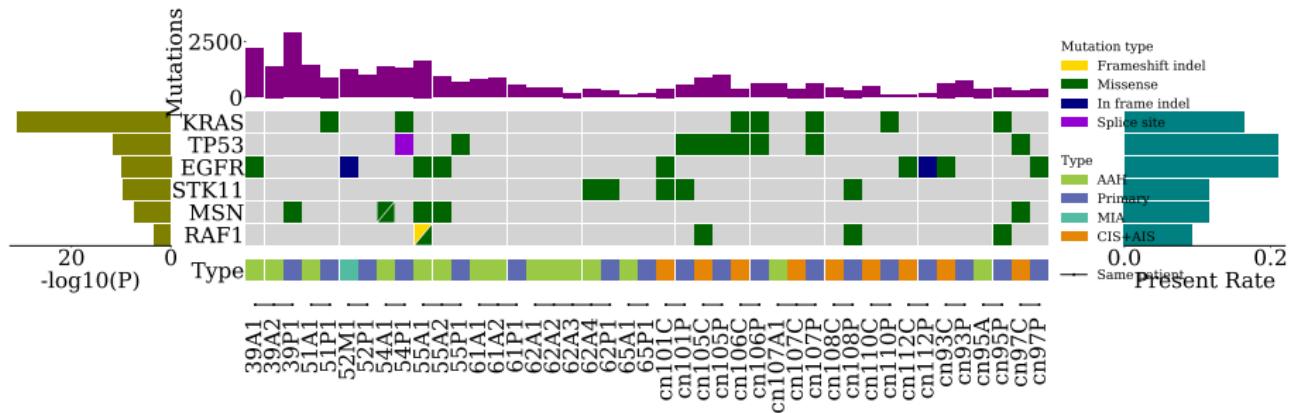


Figure: CoMut Plot with LUAD Patients

Somatic Variant in LUAD with Recurrence

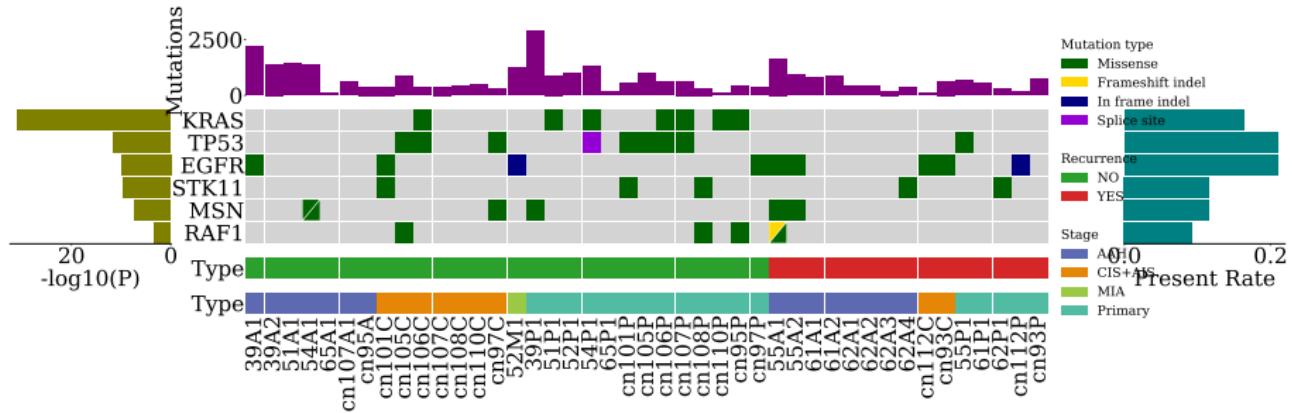


Figure: CoMut Plot in LUAD Patients with Recurrence

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

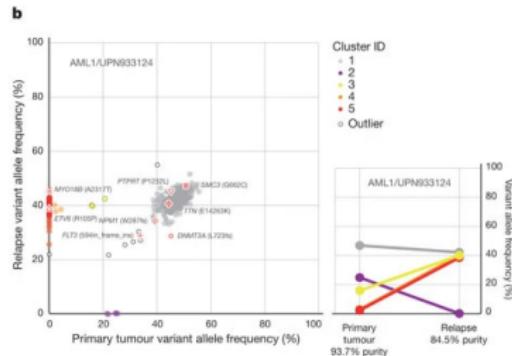
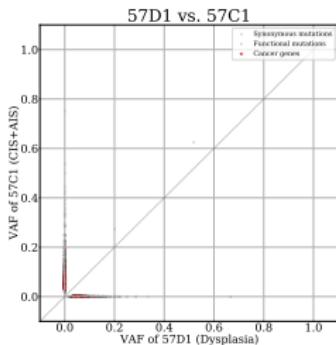
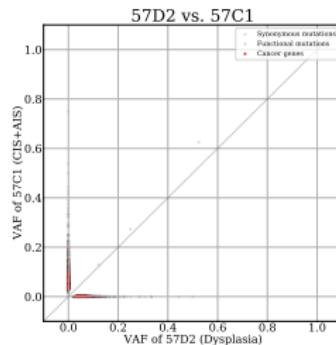


Figure: VAF distribution of validated mutations (L. Ding et al., 2012)

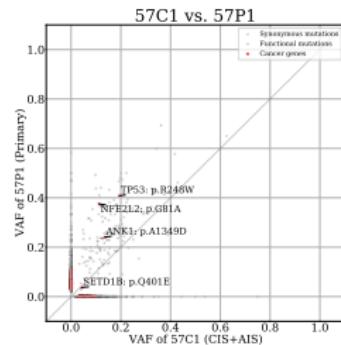
VAF Plots



(a) Dysplasia + CIS



(b) Dysplasia + CIS



(c) CIS + Primary

Figure: VAF plots in patient #57

PyClone?

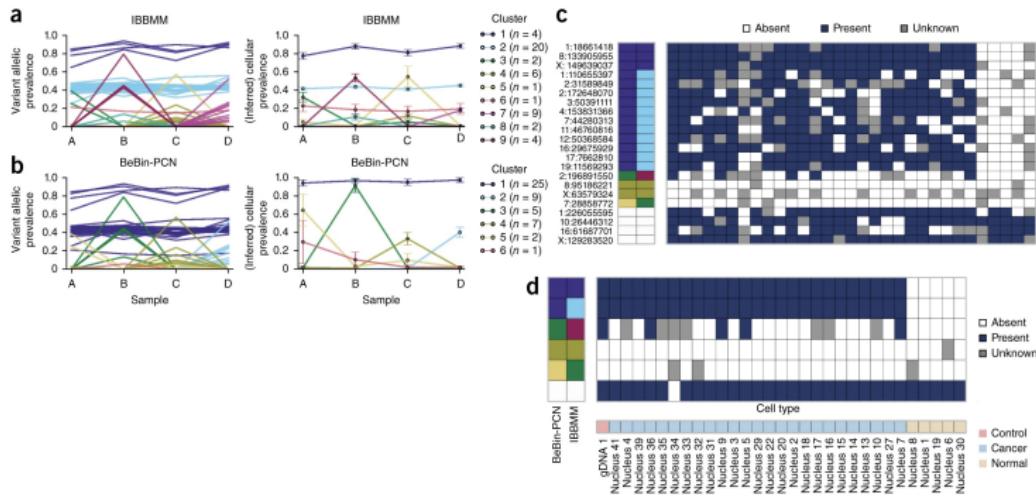
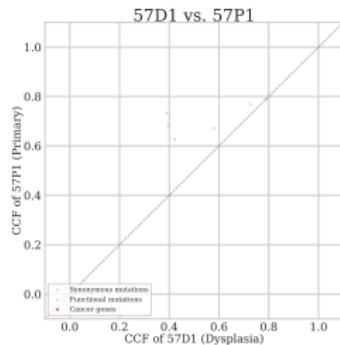
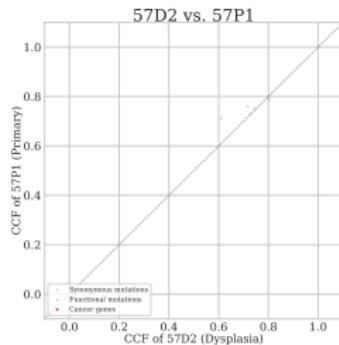


Figure: Analysis of multiple samples by PyClone (Roth et al., 2014)

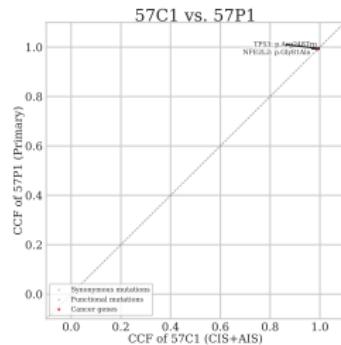
PyClone Plots I



(a) 57D1



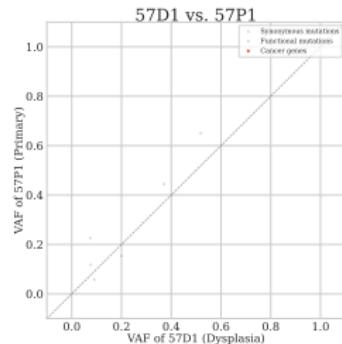
(b) 57D2



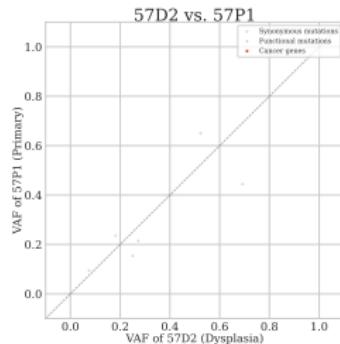
(c) 57C1

Figure: CCF plot in patient #57

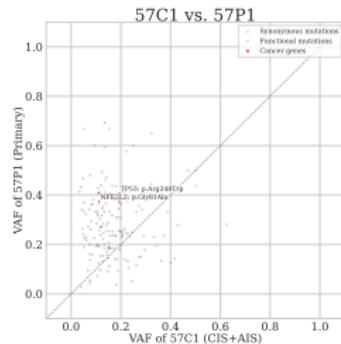
PyClone Plots II



(a) 57D1



(b) 57D2



(c) 57C1

Figure: VAF plot in patient #57

Findings in VAF Analysis

4. Results

4.6. Tumor Evolution Trajectories Analysis

Mobster?

Findings in Tumor Evolution Trajectories Analysis

4. Results

4.7. Bulk Cell Deconvolution

BisqueRNA?

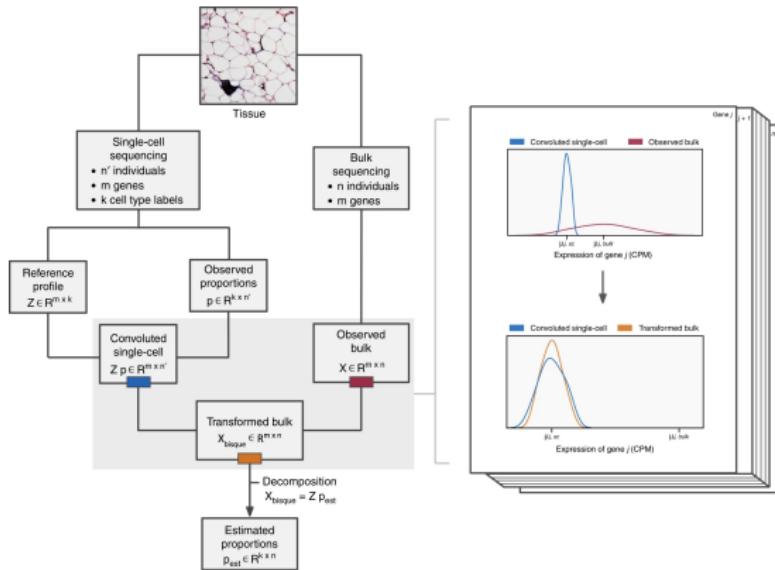


Figure: Workflow for BisqueRNA (Jew et al., 2020)

4. Results

4.7. Bulk Cell Deconvolution

4.7.1. Reference by N. Kim et al. (2020)

Reference Single-cell Data

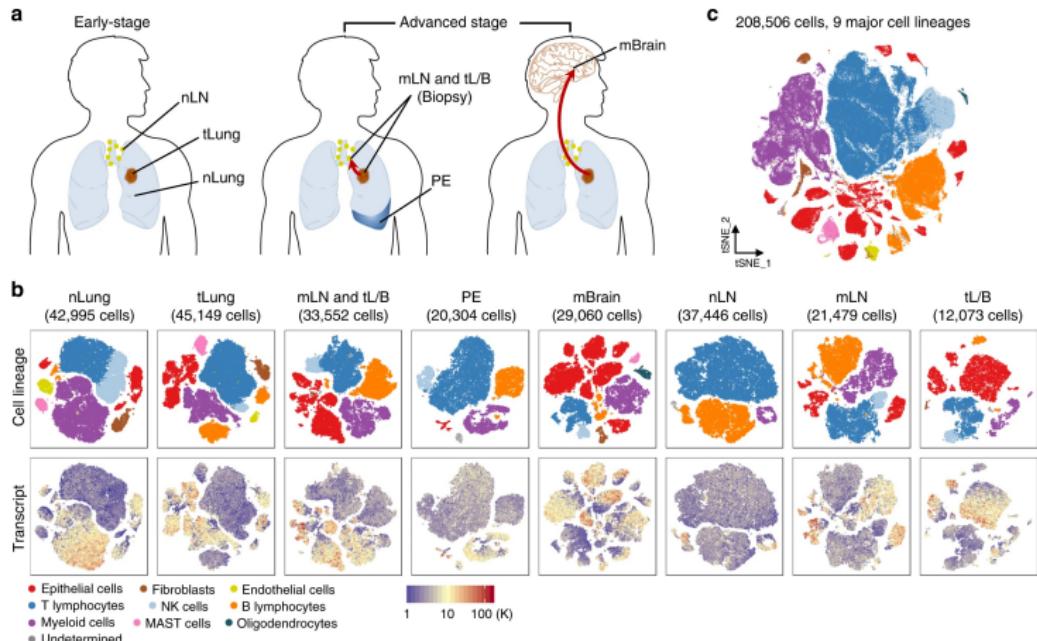


Figure: Comprehensive dissection and clustering of 208,506 single cells from LUAD patients (N. Kim et al., 2020)

Cluster Plot in LUSC

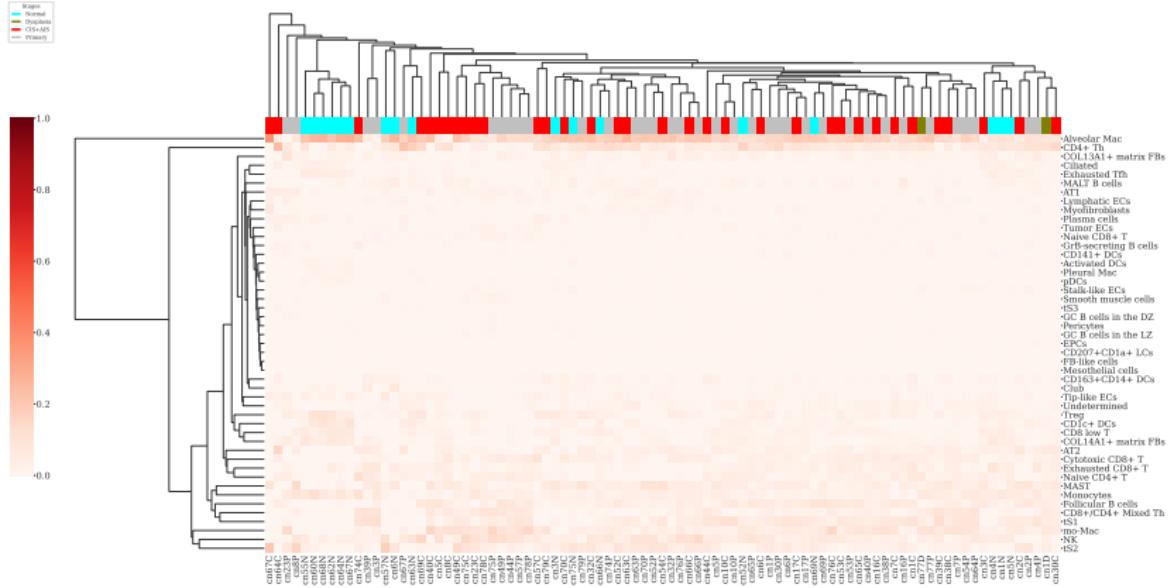
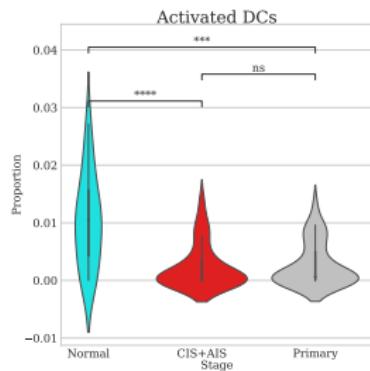
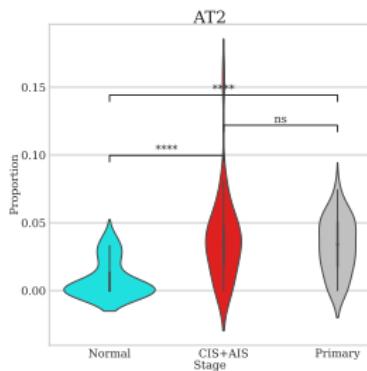


Figure: Cluster Plot in LUSC

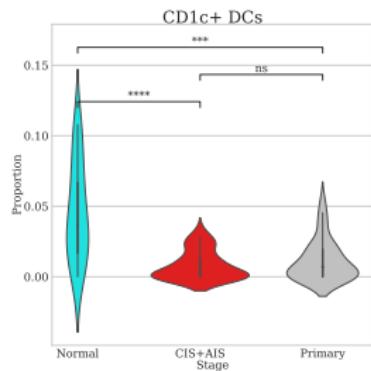
Violin Plots in LUSC I



(a) Activated DCs



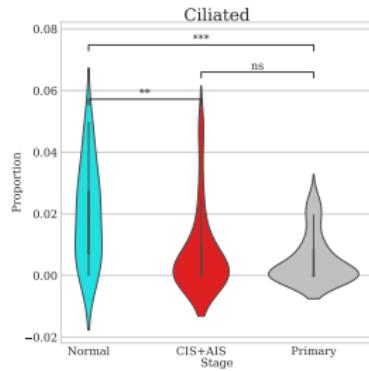
(b) Alveolar type II



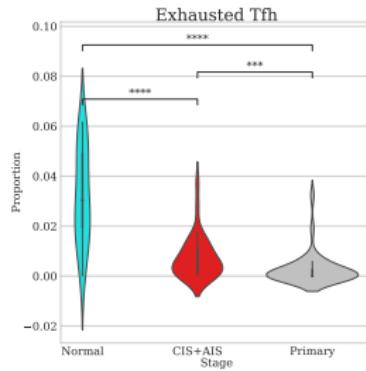
(c) Langerhans cells

Figure: Violin Plots in LUSC

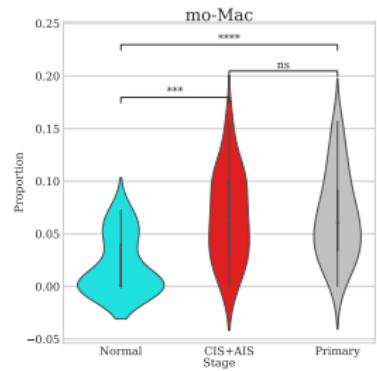
Violin Plots in LUSC II



(d) Ciliated cells



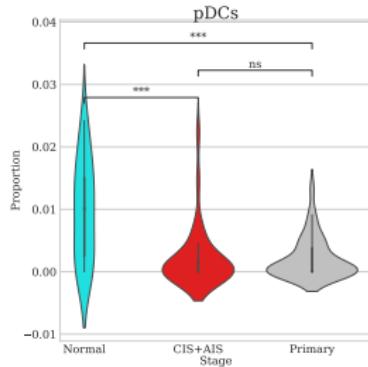
(e) Exhausted T follicular helper



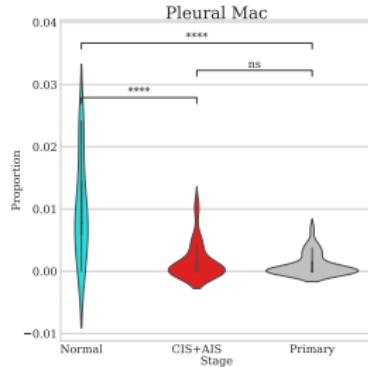
(f) Mo & Mac

Figure: Violin Plots in LUSC

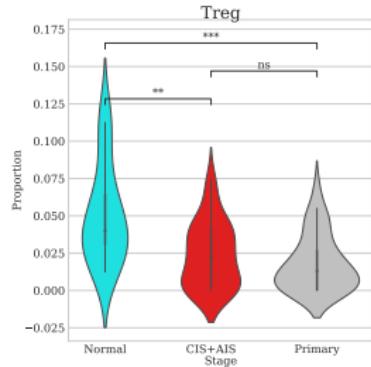
Violin Plots in LUSC III



(g) Plasmacytoid DCs



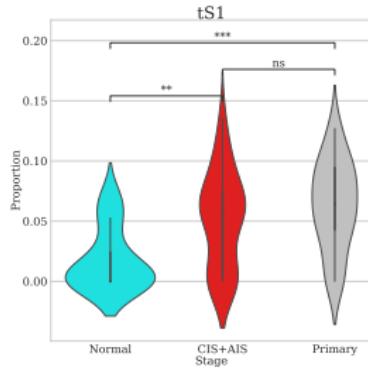
(h) Pleural Mac



(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.
- ② DCs are central regulators of adaptive immune response, e.g. anti-tumoral responses .
- ③ DCs establish a rare immune cell population in tumors .

Alveolar type II

- ① Alveolar type II have lower proportion in Normal samples.
- ② Alveolar type II proliferate to restore epithelium, and participate in innate immune response (Mason, 2006).
- ③ Capability of initiating lung cancer development (C. Lin et al., 2012).

Findings in Bulk Cell Deconvolution with LUSC II

CD1c+ DCs (Langerhans cells; LCs)

- ① LCs have higher proportion in Normal samples.
- ② LCs impact on pathology by inducing tolerance or mediating inflammation (Deckers, Hammad, & Hoste, 2018)
- ③ LCs facilitate DNA damage and squamous cell carcinoma (Modi et al., 2012)

Ciliated cells

- ① Ciliated cells have higher proportion in Normal samples.
- ② A terminally differentiated population in lung epithelial cells (Rawlins & Hogan, 2008).
- ③ Generated under homeostatic condition or response to epithelial injury (Sutherland et al., 2011).

Findings in Bulk Cell Deconvolution with LUSC III

Exhausted T follicular helper cells (Tfh)

- ① Exhausted Tfh is gradually decreased along cancer worsen.
- ② Tfh cell response is critical for viral infection (Greczmiel et al., 2017; Poonia, Ayithan, Nandi, Masur, & Kottilil, 2018a).
- ③ Down-regulated Tfh exhaustion correlate with compromise CD8 T-cell immunity (Poonia, Ayithan, Nandi, Masur, & Kottilil, 2018b)

Monocyte & Macrophage

- ① Monocyte & Macrophage have lower proportion in Normal samples.
- ② Monocyte is a regulator of tumor development & progression (Olingy, Dinh, & Hedrick, 2019).
- ③ Macrophage is a regulator of link between inflammation & cancer (Sica, Allavena, & Mantovani, 2008).

Plasmacytoid DCs (pDCs)

- ① pDCs have higher proportion in Normal samples.
- ② pDCs bring capacities of innate & adaptive immunity (Vermi, Soncini, Melocchi, Sozzani, & Facchetti, 2011).
- ③ Infiltrated pDCs in neoplasms ⇒ Poor prognosis (Pinto, Rega, Crother, & Sorrentino, 2012).

Pleural Macrophages

- ① Pleural macrophages have higher proportion in Normal samples.
- ② Neutrophil recruitment in pleural inflammation (Cailhier et al., 2006).

Findings in Bulk Cell Deconvolution with LUSC V

Regulatory T cells (Tregs)

- ① Tregs have higher proportion in Normal samples.
- ② Elevation of Tregs ↑ in solid tumors & hematologic malignancies (Beyer & Schultze, 2006).
- ③ Increasing Tregs ↑ along metastatic stage in NSCLC (Erfani et al., 2012) ??.

Tumor cell states 1 (tS1)

- ① tS1 have lower proportion in Normal samples.
- ② Represent a de-regulation of normal differentiation programs (N. Kim et al., 2020).

Cluster Plot in LUAD

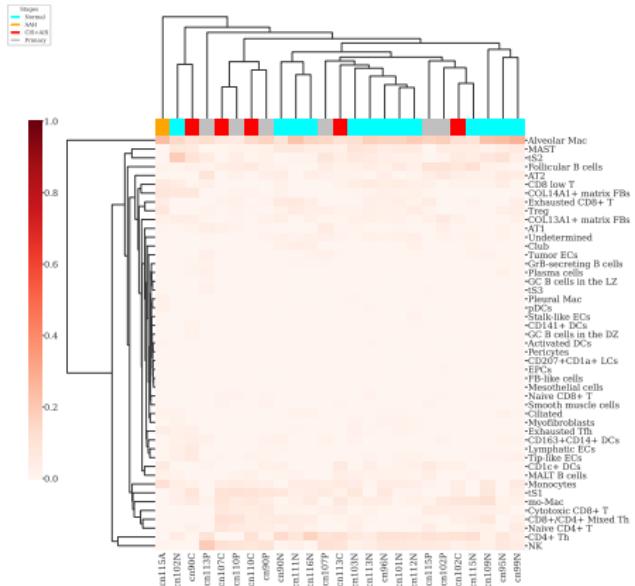
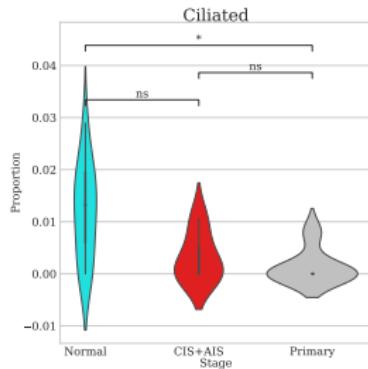
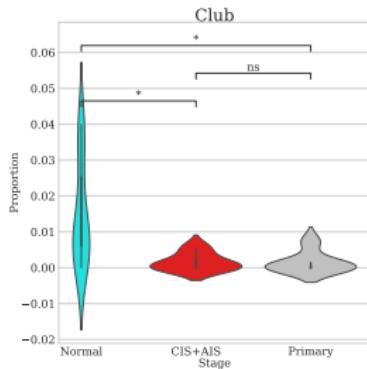


Figure: Cluster Plot in LUAD

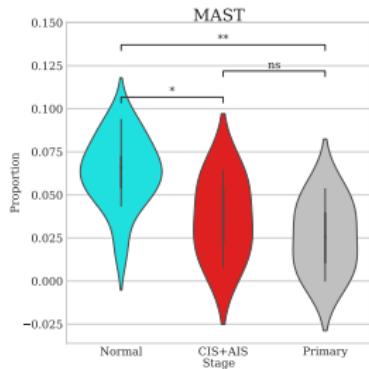
Violin Plots in LUAD I



(a) Ciliated cells



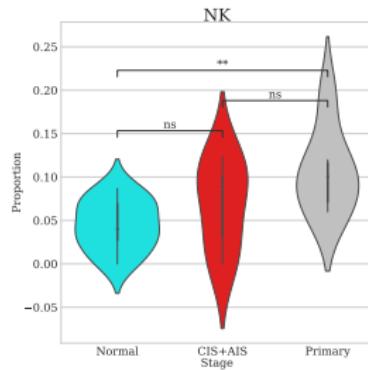
(b) Club Cell



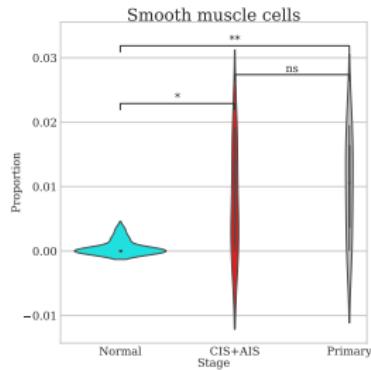
(c) Mast cell

Figure: Violin Plots in LUAD

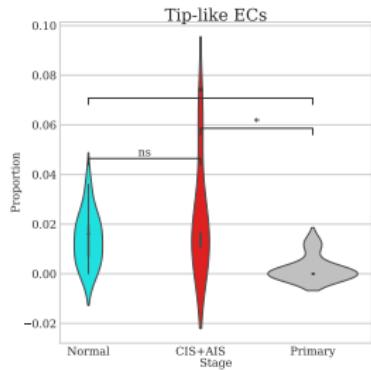
Violin Plots in LUAD II



(d) NK cells



(e) Smooth muscle cells



(f) Tip-like ECs

Figure: Violin Plots in LUAD

Ciliated cells

- ① Ciliated cells have higher proportion in Normal than Primary samples.
- ② A terminally differentiated population in lung epithelial cells (Rawlins & Hogan, 2008).
- ③ Generated under homeostatic condition or response to epithelial injury (Sutherland et al., 2011).

Findings in Bulk Cell Deconvolution with LUAD II

Club cells

- ① Club cells have higher proportion in Normal than Primary samples.
- ② Club cells form LUAD in adult mice (Spella et al., 2019).
- ③ Club cells in smoking-associated LUAD (Behrend, Giotopoulou, Spella, & Stathopoulos, 2021).
- ④ Increasing club cells ⇒ Good indicator of advanced bronchopulmonary dysplasia (Rokicki, Rokicki, Wojtacha, & Dżelijjli, 2016).

Mast cells

- ① Mast cells have higher proportion in Normal than Primary samples.
- ② Mast cells activated by lung cancer-derived extracellular vesicles (Xiao et al., 2019).
- ③ Mast cell promote ↑ tumor metastasis (Salamon, Mekori, & Shefler, 2020).

Natural Killer cells (NK cells)

- ① NK cells have higher proportion in Primary than Normal samples.
- ② NK cells play a major role in innate immune system (Shin et al., 2020).
- ③ NK cells can induce immune response against tumor cells (Shin et al., 2020).
- ④ NK cells may induce tumor regression in lung cancer (Aktaş et al., 2018) ??.

Findings in Bulk Cell Deconvolution with LUAD IV

Smooth muscle cells

- ① Smooth muscle cells have higher proportion in Primary than Normal samples.
- ② Hypoxia is a characteristic feature of solid tumors (Brahimi-Horn, Chiche, & Pouysségur, 2007; Vaupel & Mayer, 2007).
- ③ ∴ Smooth muscle cells pathway is up-regulated in cancer (Kyotani, Takasawa, & Yoshizumi, 2019; T.-T. Zhu et al., 2019).

Tip-like endothelial cells (ECs)

- ① Tip-like ECs have lower proportion in Primary than Normal samples.
- ② Tip-like ECs were determined into migratory & basement-membrane remodeling phenotypes (Goveia et al., 2020).
- ③ Tip-like ECs replaced with immature ones in NSCLC (E. Y. Kim et al., 2022).

4. Results

4.7. Bulk Cell Deconvolution

4.7.2. Reference by Gueguen et al. (2021)

Reference Single-cell Data

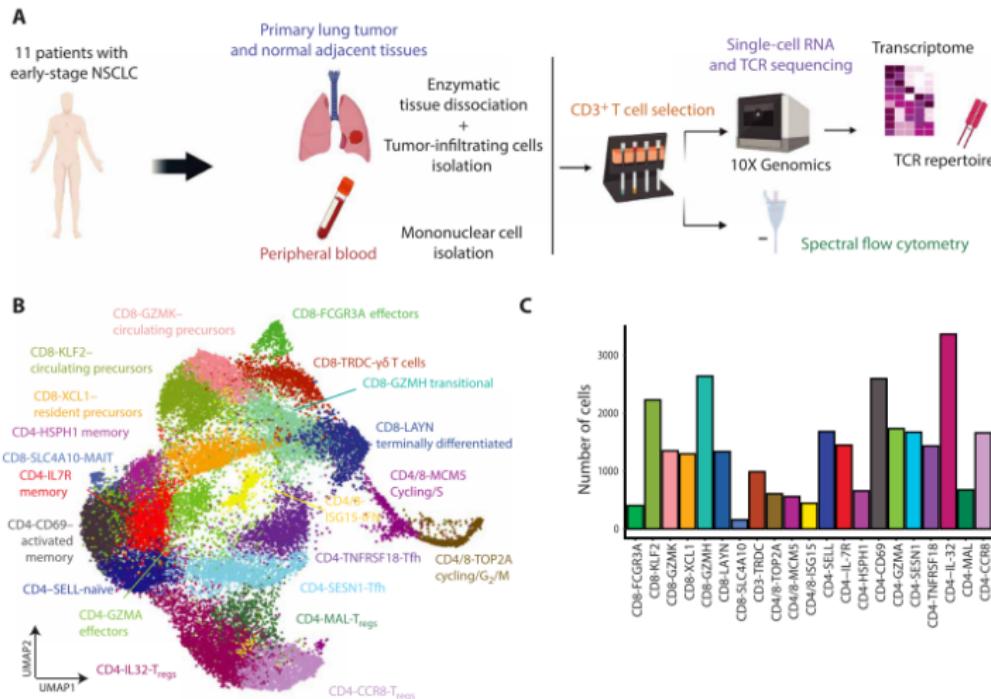


Figure: Characterization of CD3⁺ TILs in NSCLC (Gueguen et al., 2021)

Cluster Plots in LUSC

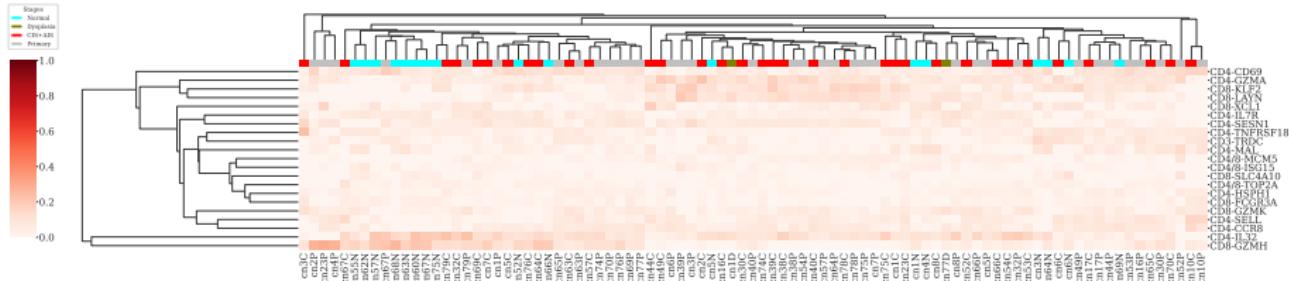


Figure: Cluster Plot in LUAD

Violin Plots in LUSC I

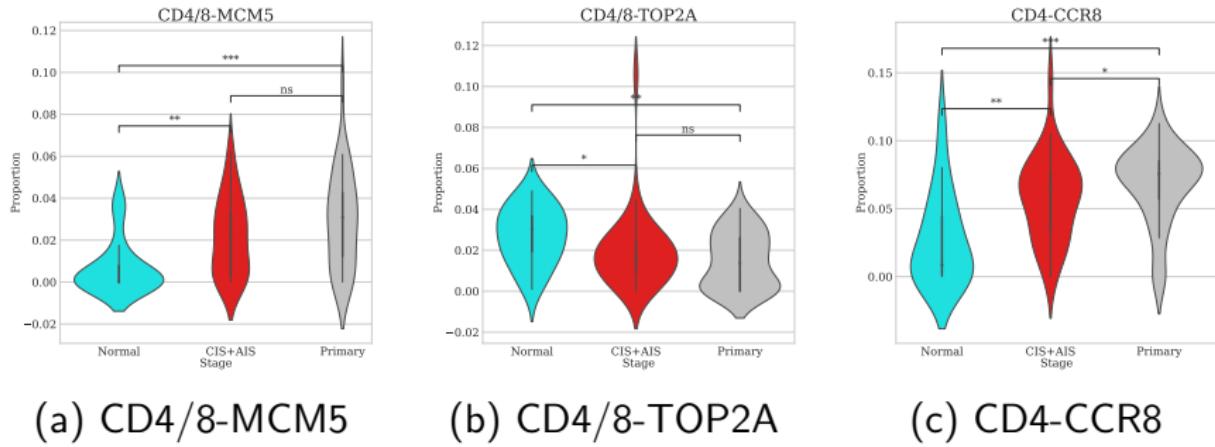
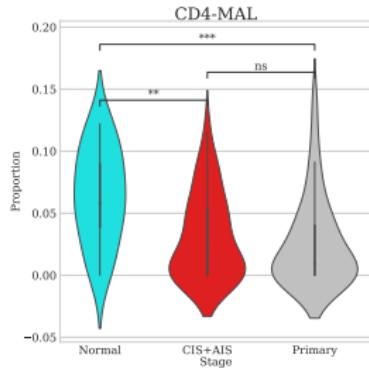
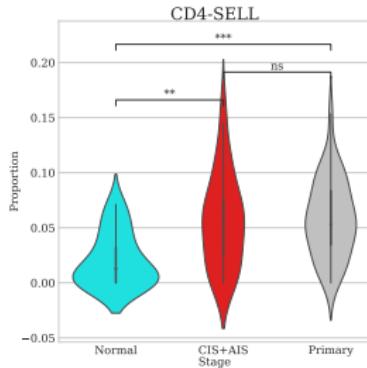


Figure: Violin Plots in LUSC

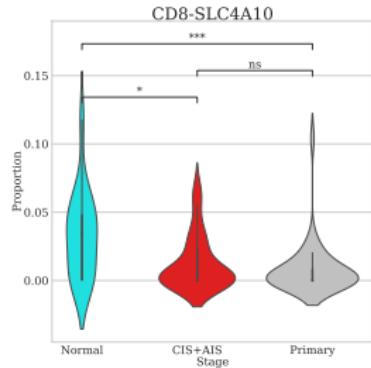
Violin Plots in LUSC II



(d) CD4-MAL



(e) CD4-SELL



(f) CD8-SLC4A10

Figure: Violin Plots in LUSC

CD4/8-MCM5

- ① CD4/8-MCM5 have lower proportion in Normal.
- ② MCM5, mini-chromosome maintenance protein 5, acts as component of MCM complex (Tsuiji, Ficarro, & Jiang, 2006).
- ③ MCM5, thus, play a major role in replication and cell cycle progression (Paul, Hu, Musahl, Hameister, & Knippers, 1996).
- ④ MCM5 could be adverse prognostic marker for NSCLC (Grzegrzolka et al., 2021) and lung cancer (Y.-Z. Liu et al., 2017).

Findings in Bulk Cell Deconvolution with LUSC II

CD4/8-TOP2A

- ① CD4/8-TOP2A have higher proportion in Normal.
- ② TOP2A, topoisomerase IIA, have an essential role for modulating DNA topology & cell division (Wyles, Wu, Mirski, & Cole, 2007).
- ③ TOP2A over-expressed ↑ in bladder cancer (Zeng et al., 2019), LUAD (Kou et al., 2020) and NSCLC (W. Ma et al., 2019) ??

CD4-CCR8

- ① CD4-CCR8 is gradually increased along tumor progression.
- ② CCR8, C-C chemokine receptor type 8, might modulate monocyte chemotaxis and tymic cell line apoptosis (Tiffany et al., 1997).
- ③ CCR8 up-regulated along tumor progression in bladder (X. Liu et al., 2019), colon (Villarreal et al., 2018), and breast cancer (Plitas et al., 2016).

CD4-MAL

- ① CD4-MAL have lower proportion in Primary.
- ② MAL, myelin and lymphocyte protein, play a role in indirect route for egress of transcytosing cargo (de Marco et al., 2002).
- ③ Over-expression of MAL was correlated with worse prognostic factors in uterine carcinoma (D. Li et al., 2021).
- ④ MAL was highly methylated in gastric cancer (Choi et al., 2017).
- ⑤ MAL acts as a tumor suppressor or a tumor progression factor among cancer types (Lara-Lemus, 2019).

Findings in Bulk Cell Deconvolution with LUSC IV

CD4-SELL

- ① CD4-SELL have higher proportion in Primary.
- ② SELL, a calcium-dependent lectin, controls cell adhesion with neighboring cells (Bernimoulin et al., 2003; Wedepohl et al., 2017).
- ③ SELL over-expressed in breast cancer (Kumari et al., 2021).

CD8-SLC4A10

- ① CD8-SLC4A10 have lower proportion in Primary.
- ② SLC4A10, sodium-driven chloride bicarbonate exchanger, have an essential role in regulating intracellular pH (C.-Z. Wang, Yano, Nagashima, & Seino, 2000).
- ③ SLC4A10 disruption leads to an extreme change in the cellular phenotype (Christensen et al., 2020).

Cluster Plots in LUAD

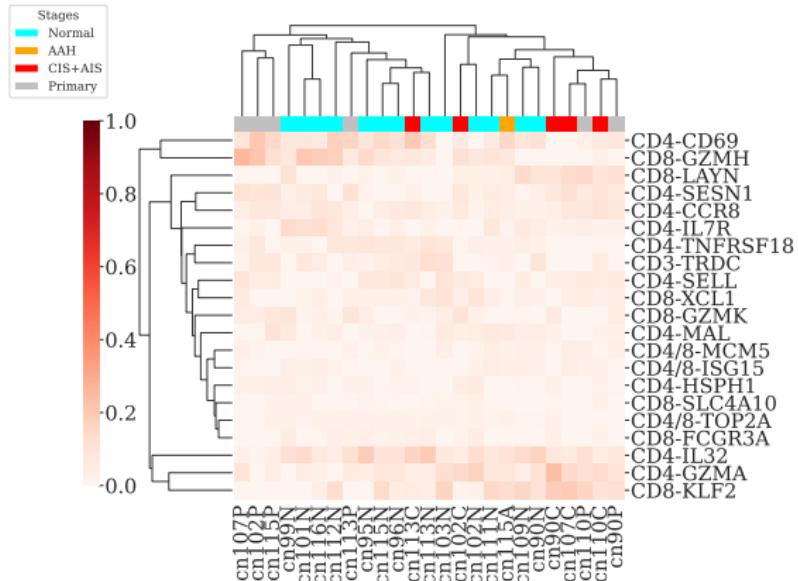
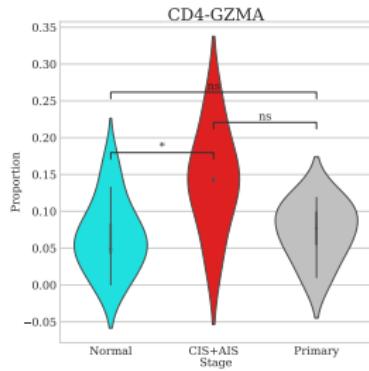
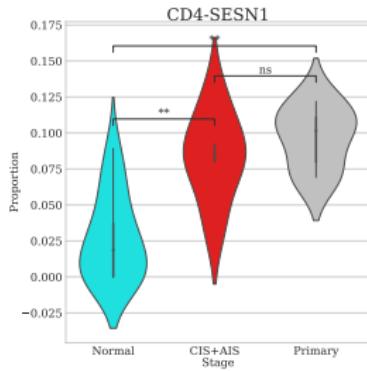


Figure: Cluster Plot in LUAD

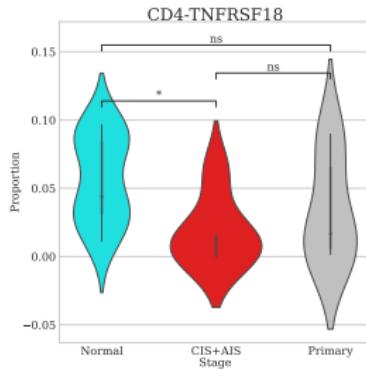
Violin Plots in LUAD



(a) CD4-GZMA



(b) CD4-SESN1



(c) CD4-TNFRSF18

Figure: Violin Plots in LUAD

CD4-GZMA

- ① CD4-GZMA have higher proportion in AIS than Normal.
- ② GZMA, granzyme A, activates caspase-independent pyroptosis through the immunological synapse (Gershenson, Hershberger, Shows, & Weissman, 1988; Hameed, Lowrey, Lichtenheld, & Podack, 1988; Krähenbühl et al., 1988).
- ③ GZMA promotes many cancers.
 - colorectal cancer (Santiago et al., 2020; Narayanan et al., 2018)
 - breast cancer (Fisler, Sikaria, Yavorski, Tu, & Blanck, 2018)
 - NSCLC (Jia et al., 2018)

CD4-SESN1

- ① CD4-SESN1 have higher proportion in AIS than Normal.
- ② SESN1, sestrin-1, acts as an intracellular *leucine* sensor that controls the TORC1 signaling pathway (Chantranupong et al., 2014; Wolfson et al., 2016).
- ③ SESN1 is controlled by p53 tumor suppressor, and thus affects in cell growth regulation (Budanov & Karin, 2008).
- ④ Inhibitor of SESN1 implicates to the pro-oxidant and oncogenic effects of mutant p53 (Cordani et al., 2018)?
- ⑤ SESN1 plays opposite role in *early* and *late* stage of lung carcinogenesis (B. Ding et al., 2019).

CD4-TNFRSF18

- ① CD4-TNFRSF18 have lower proportion in AIS than Normal.
- ② TNFRSF18, tumor necrosis factor receptor super-family member 18, is a receptor for TNFSF18.
- ③ TNFSF18 regulates T-cell responses, and promotes ↑ leukocyte adhesion to endothelial cells (Lacal et al., 2013).
- ④ TNFRSF18 was negatively correlated with survival in endometrial cancer (Zhou, Zhang, Li, Chen, & Cheng, 2020)?

Findings in Bulk Cell Deconvolution

4. Results

4.8. Discovery of Mutational Signature

Mutational Signature?

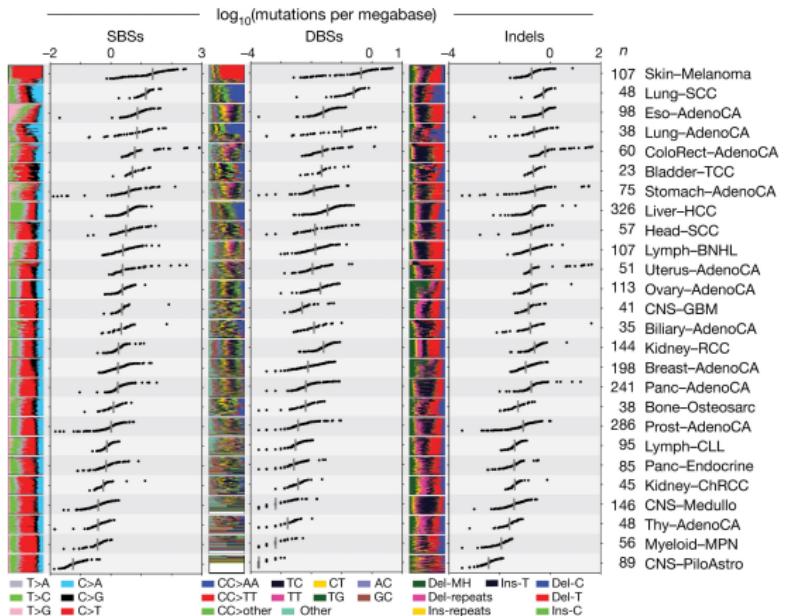


Figure: Mutation Burdens across PCAWG tumor types (Alexandrov et al., 2020)

SigProfiler?

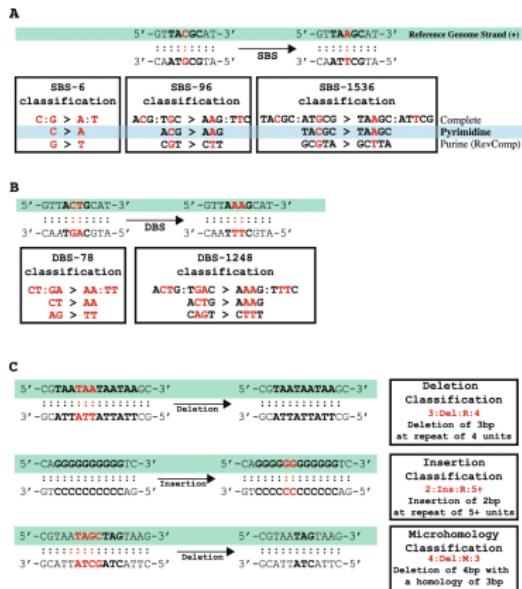


Figure: Classification of mutation signatures by SigProfiler (Bergstrom et al., 2019; Islam et al., 2021; Bergstrom et al., 2020)

4. Results

4.8. Discovery of Mutational Signature

4.8.1. Single Base Substitutions (SBS)

SBS Signatures I

SBS1

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

SBS2

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

SBS4

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

SBS5

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

SBS10b

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

SBS Signatures III

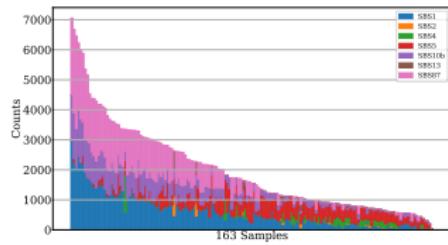
SBS13

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

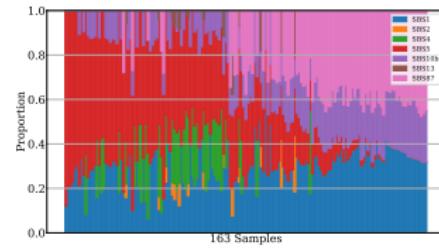
SBS87

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

SBS in LUSC I



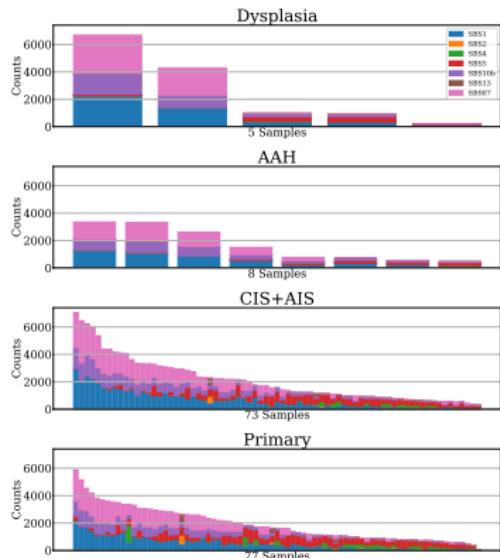
(a) Absolute



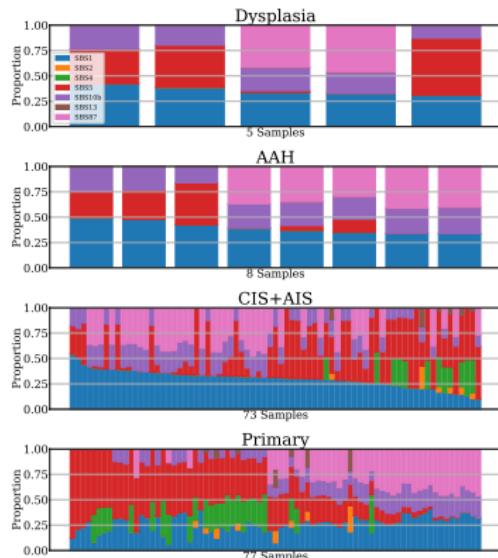
(b) Relative

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

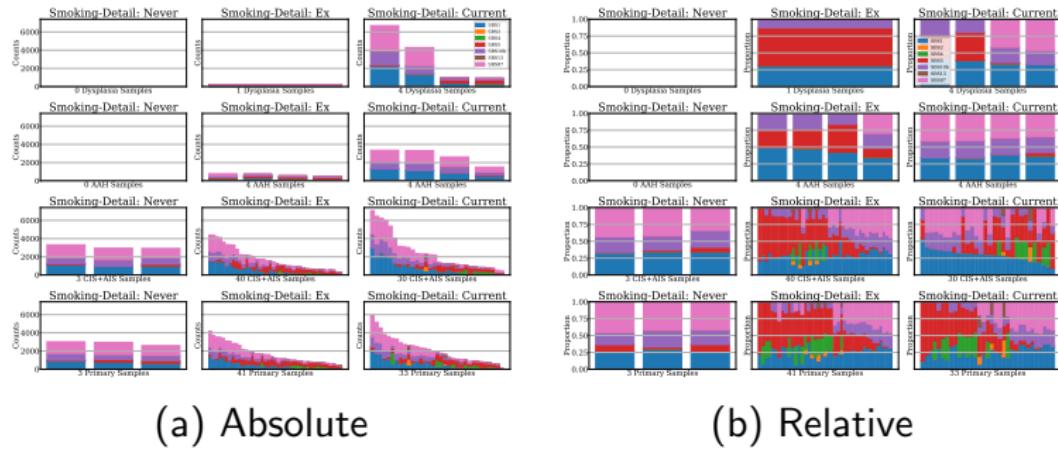
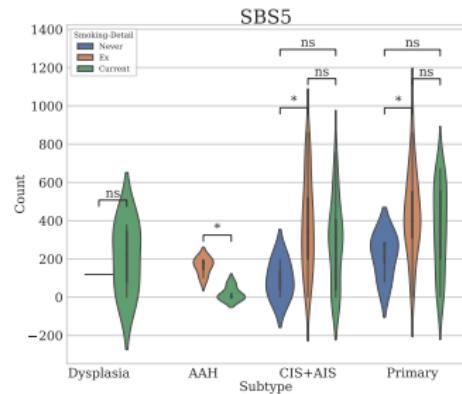
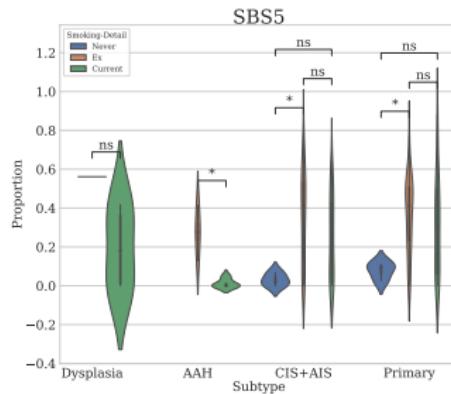


Figure: SBS Bar Plot by Cancer Subtype & Smoking in LUSC

SBS in LUSC with Smoking II



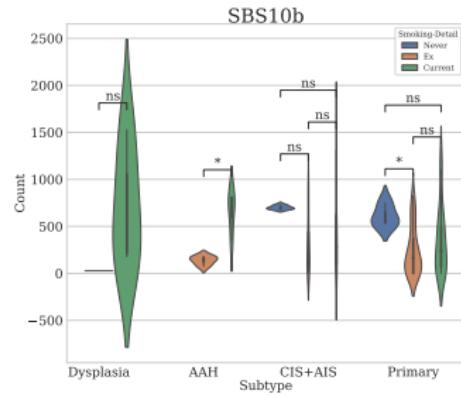
(a) Absolute



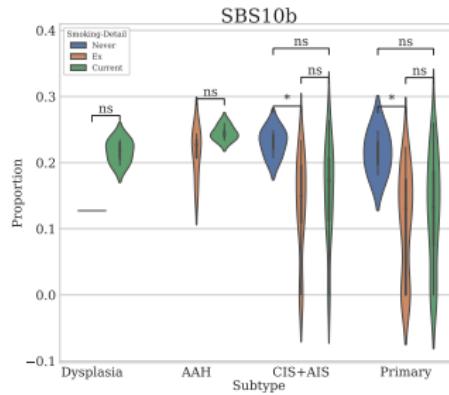
(b) Relative

Figure: SBS5 Signature in LUSC with Smoking

SBS in LUSC with Smoking III



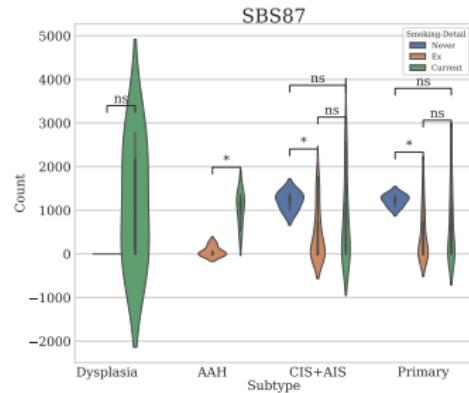
(a) Absolute



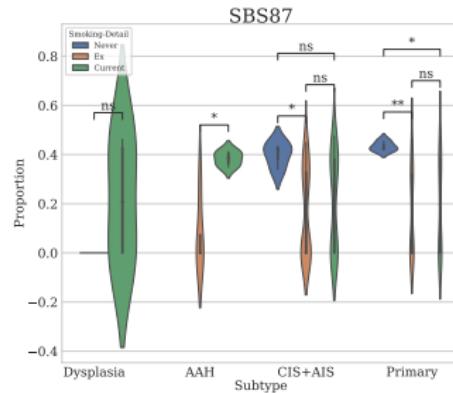
(b) Relative

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

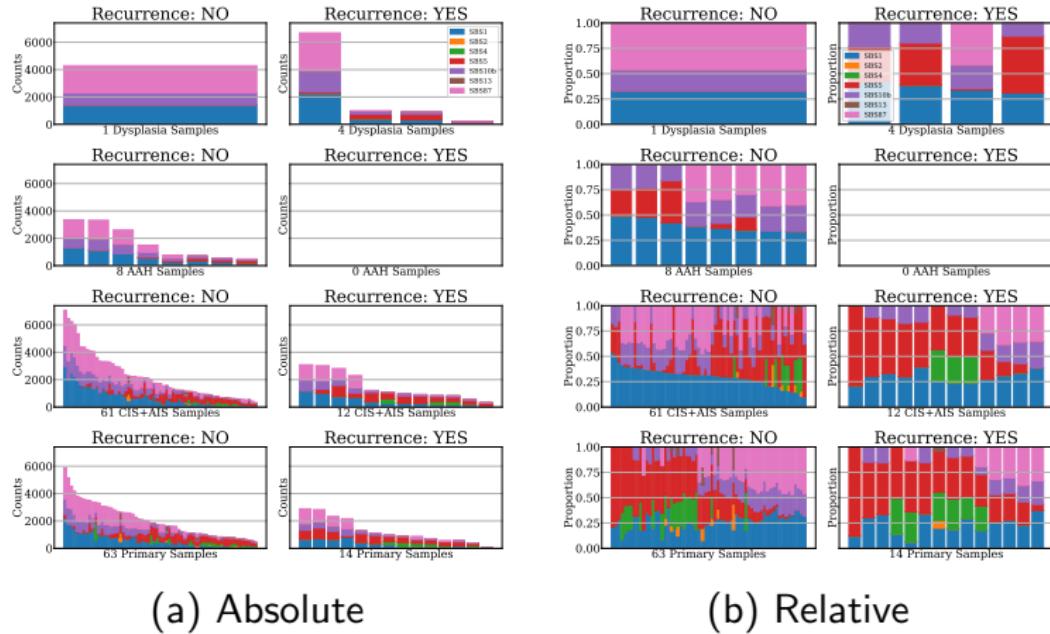
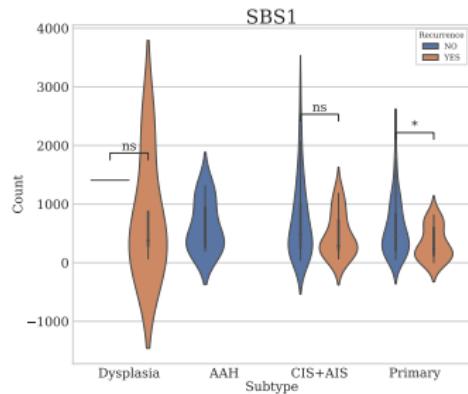
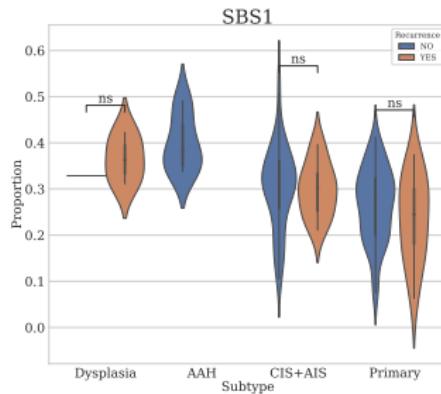


Figure: SBS Bar Plot by Cancer Subtype & Recurrence in LUSC

SBS in LUSC with Recurrence II



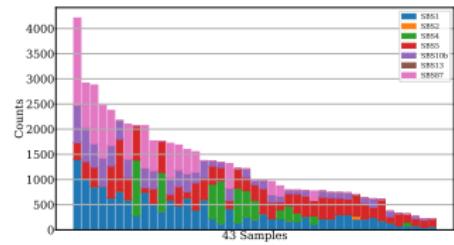
(a) Absolute



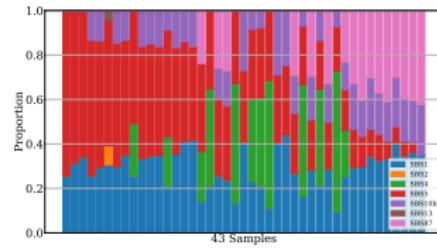
(b) Relative

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

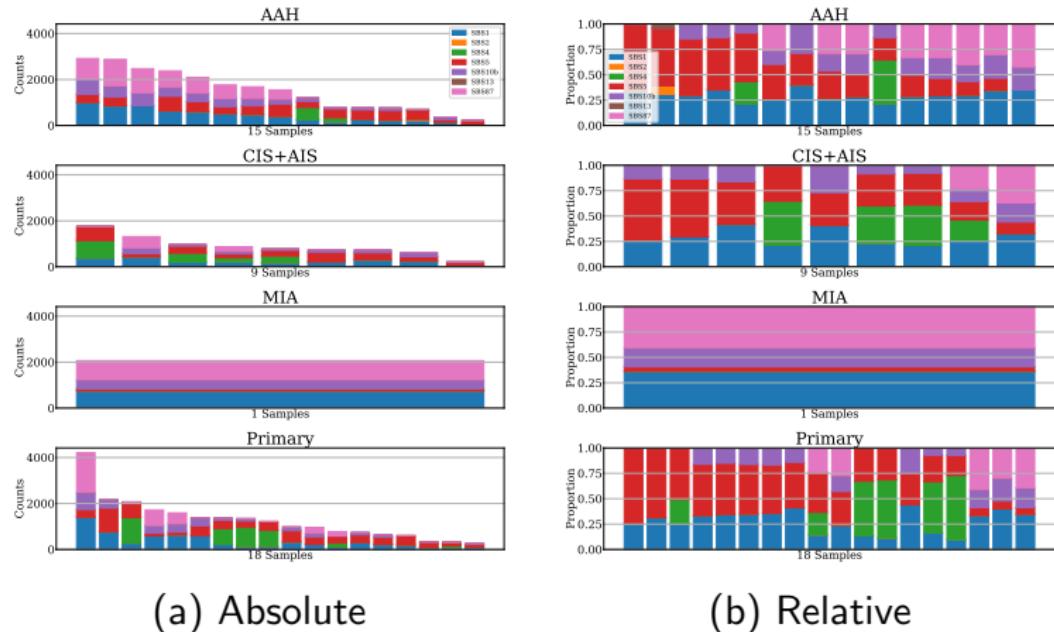


Figure: SBS Bar Plot by Cancer Subtype in LUSC

SBS in LUAD with Smoking I

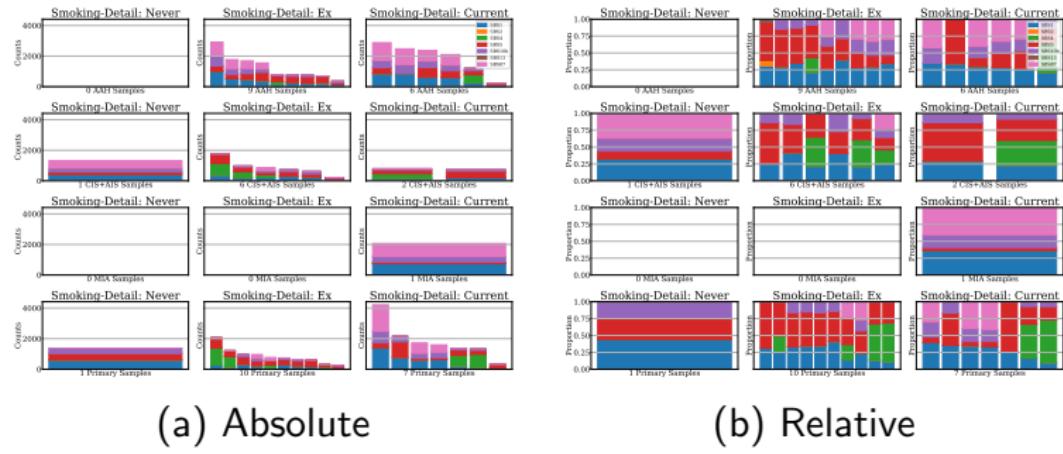
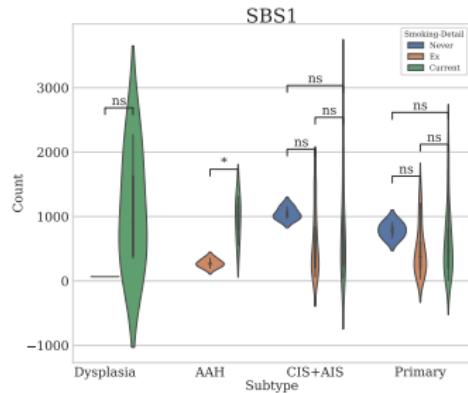
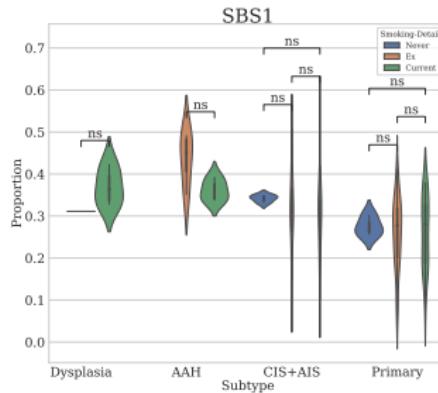


Figure: SBS Bar Plot by Cancer Subtype & Smoking in LUAD

SBS in LUAD with Smoking II



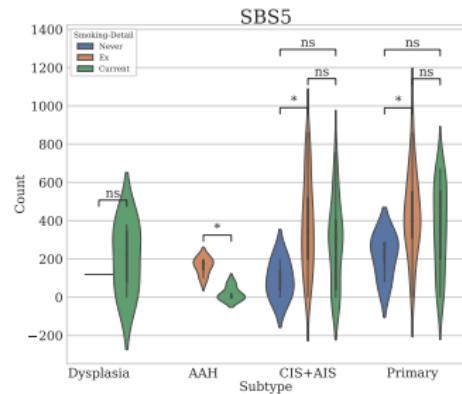
(a) Absolute



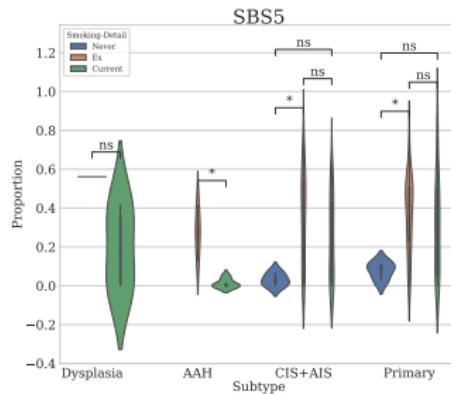
(b) Relative

Figure: SBS1 Signature in LUAD with Smoking

SBS in LUAD with Smoking III



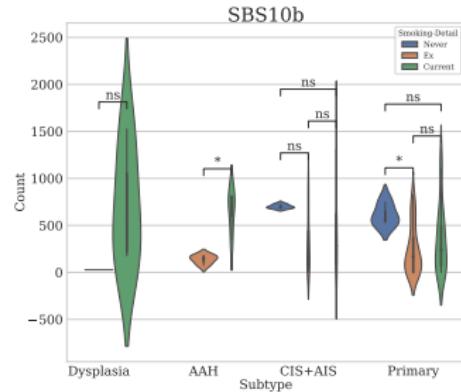
(a) Absolute



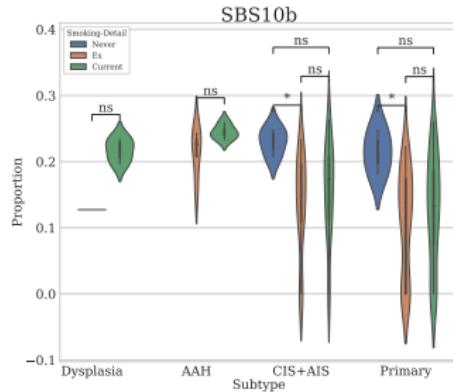
(b) Relative

Figure: SBS5 Signature in LUAD with Smoking

SBS in LUAD with Smoking IV



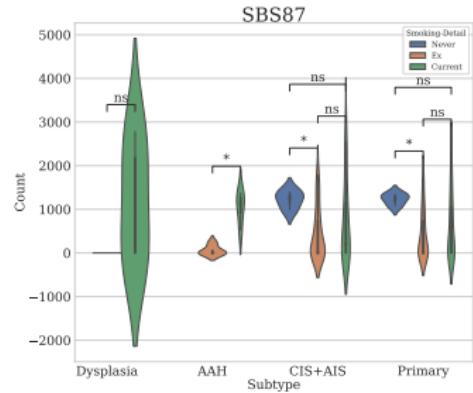
(a) Absolute



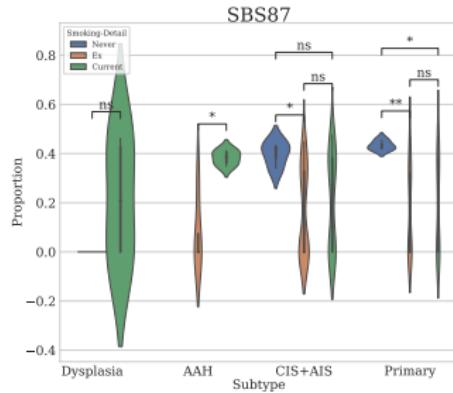
(b) Relative

Figure: SBS10b Signature in LUAD with Smoking

SBS in LUAD with Smoking V



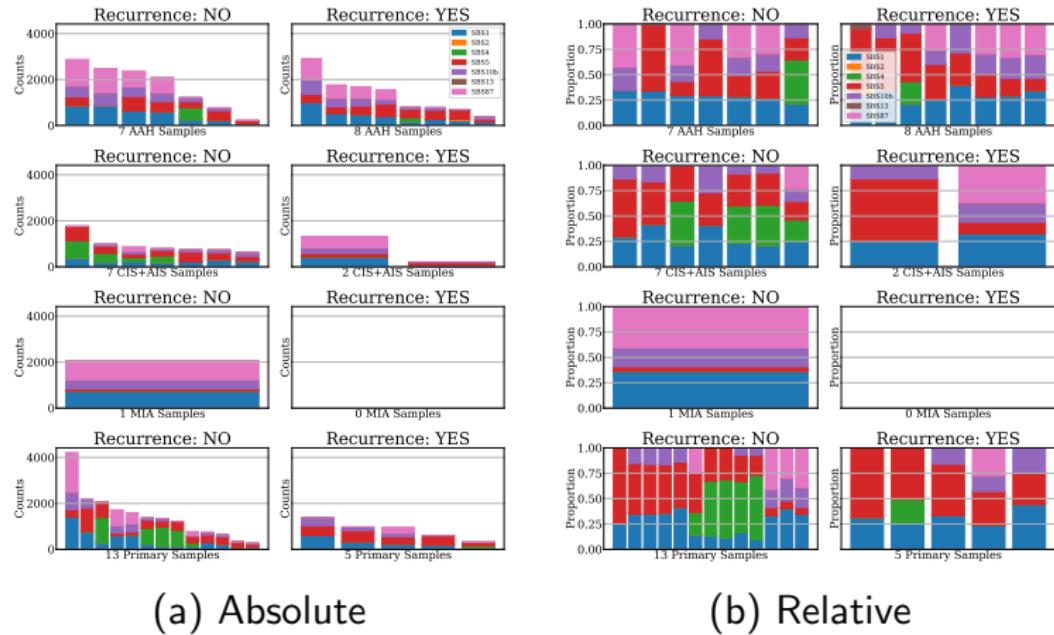
(a) Absolute



(b) Relative

Figure: SBS87 Signature in LUAD with Smoking

SBS in LUAD with Recurrence I

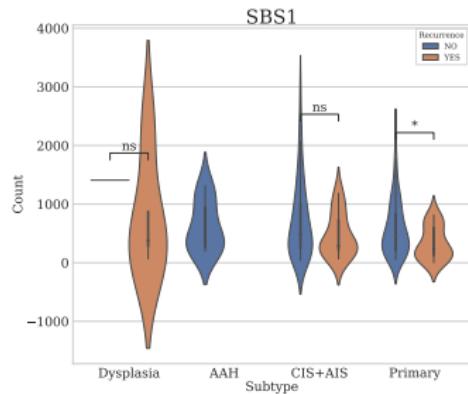


(a) Absolute

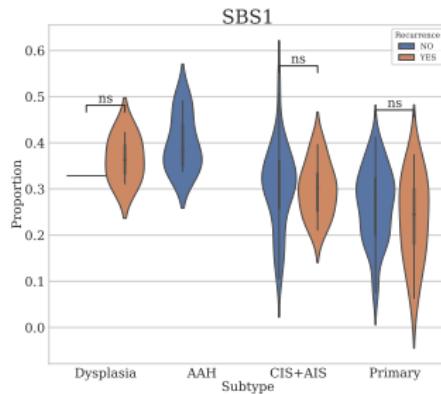
(b) Relative

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

4.8. Discovery of Mutational Signature

4.8.2. Double Base Substitutions (DBS)

DBS Signatures I

DBS2

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

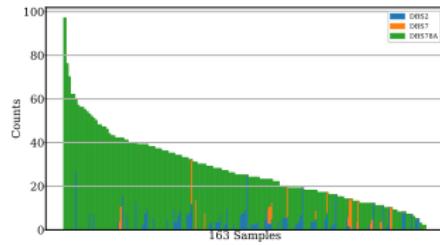
DBS7

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

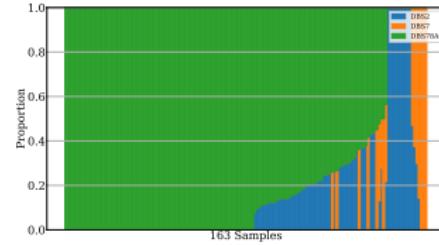
DBS78A

content...

DBS in LUSC I

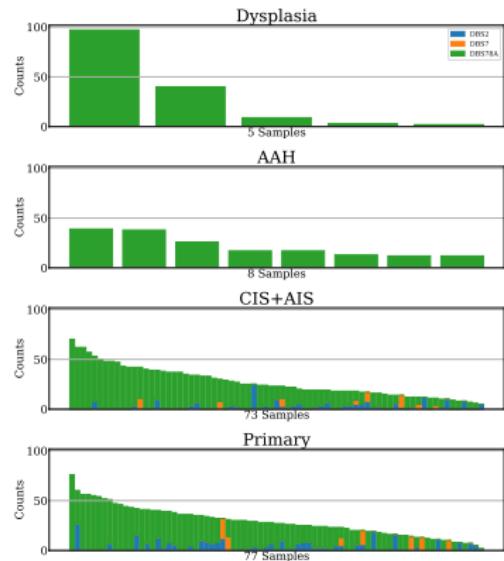


(a) Absolute

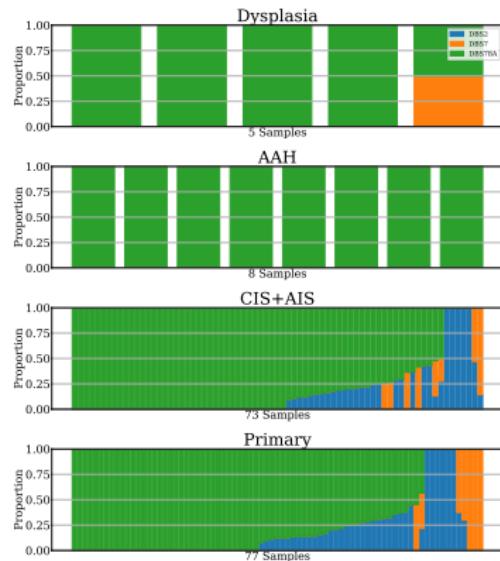


(b) Relative

DBS in LUSC II



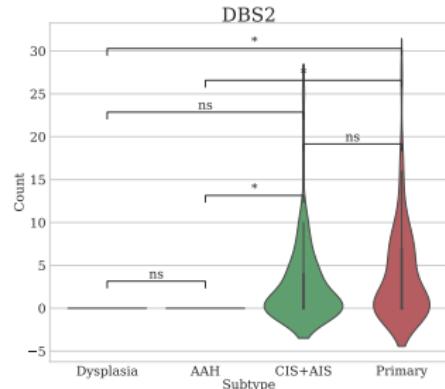
(a) Absolute



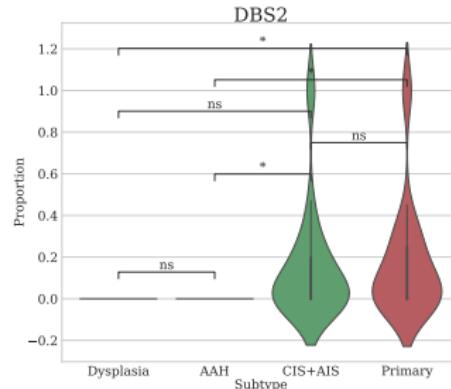
(b) Relative

Figure: DBS Bar Plot by Cancer Subtype in LUSC

DBS in LUSC III



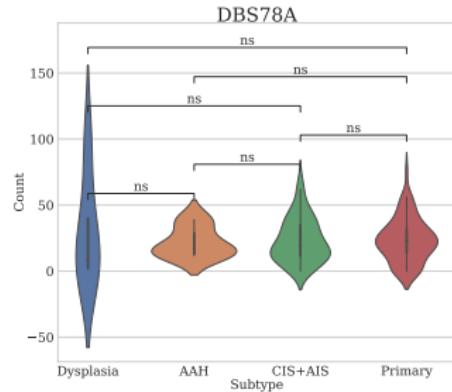
(a) Absolute



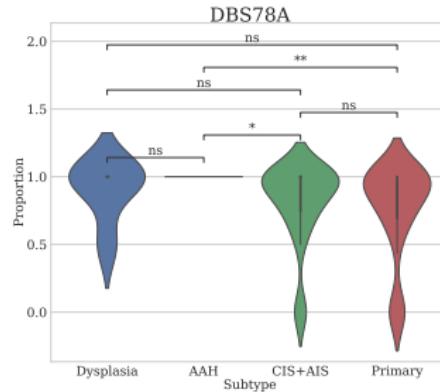
(b) Relative

Figure: DBS2 Signature in LUSC

DBS in LUSC IV



(a) Absolute



(b) Relative

Figure: DBS78A Signature in LUSC

DBS in LUSC with Smoking I

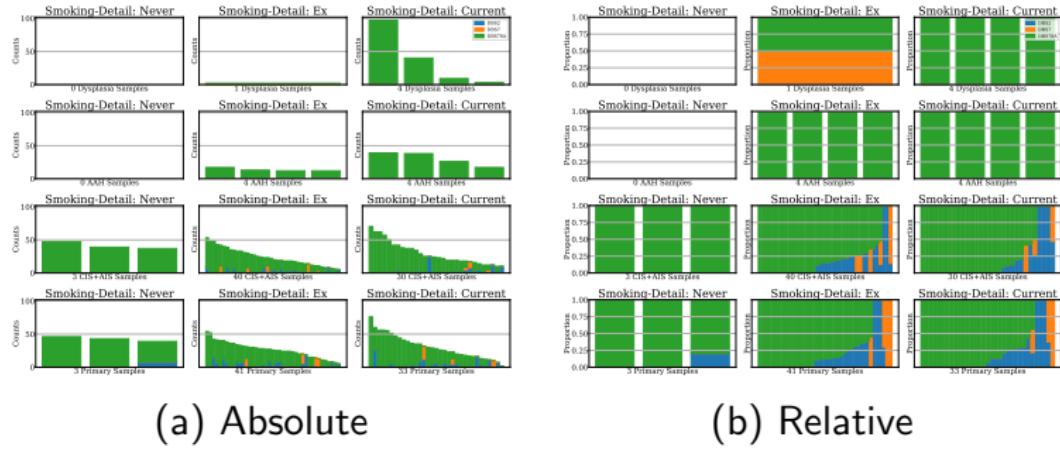
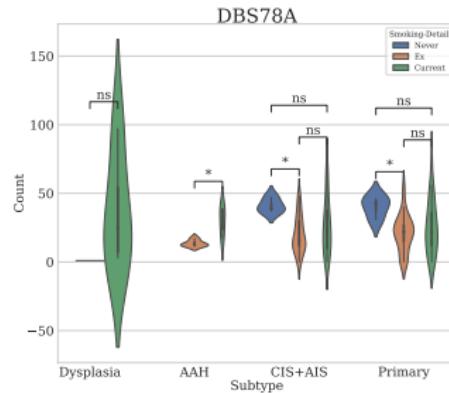
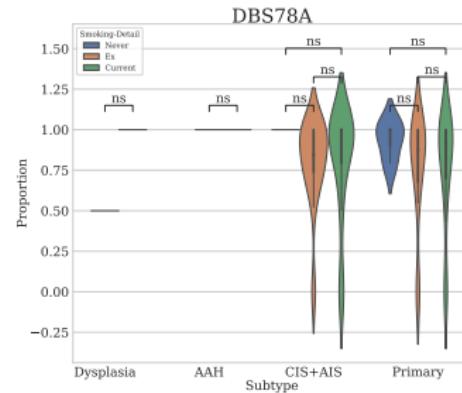


Figure: DBS Bar Plot by Cancer Subtype & Smoking in LUSC

DBS in LUSC with Smoking II



(a) Absolute



(b) Relative

Figure: DBS78A Signature in LUSC with Smoking

DBS in LUSC with Recurrence

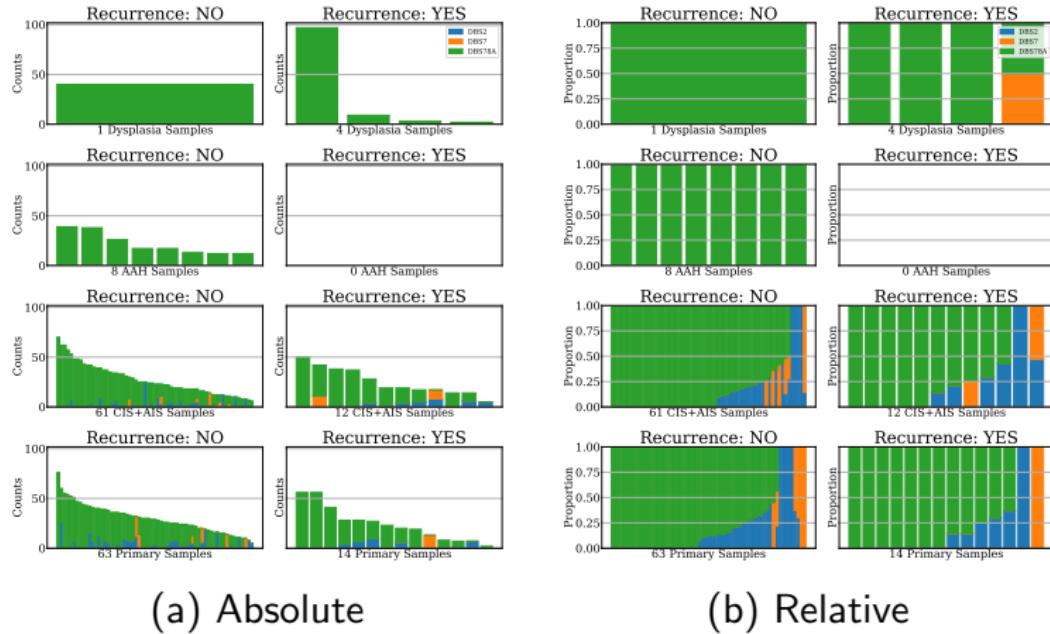
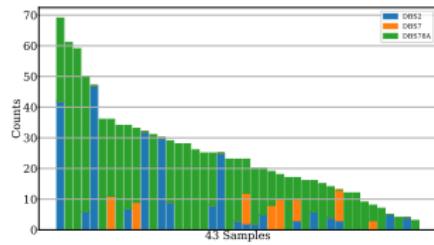
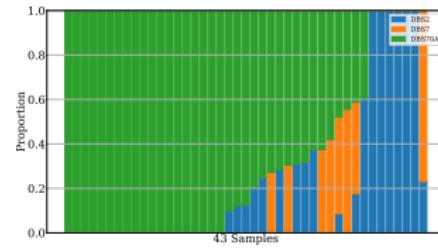


Figure: DBS Bar Plot by Cancer Subtype & Recurrence in LUSC

DBS in LUAD I



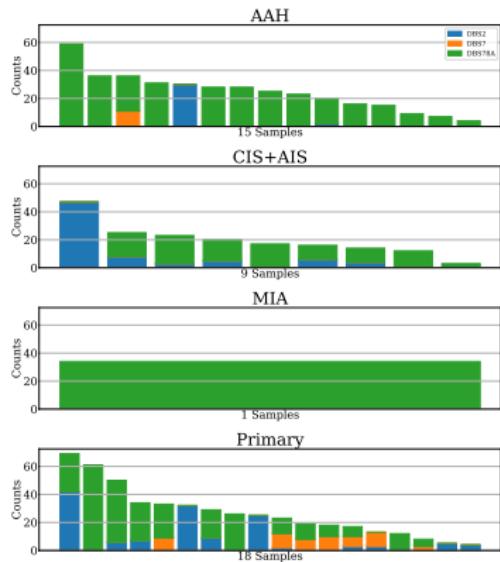
(a) Absolute



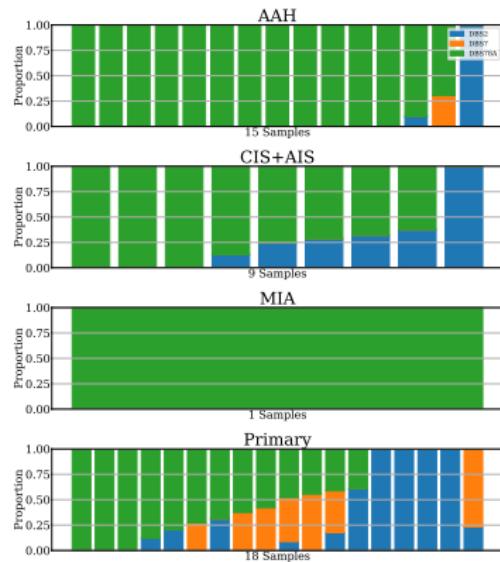
(b) Relative

Figure: DBS Bar Plot in LUAD

DBS in LUAD II



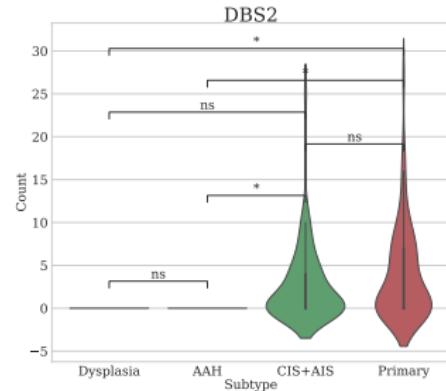
(a) Absolute



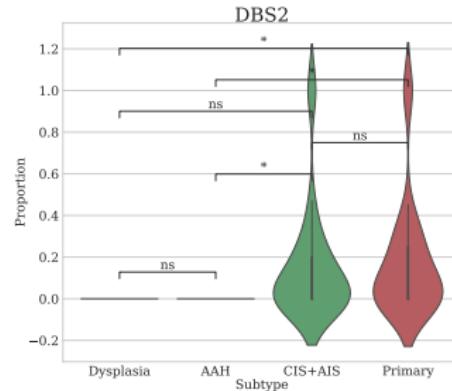
(b) Relative

Figure: DBS Bar Plot by Cancer Subtype in LUAD

DBS in LUAD III



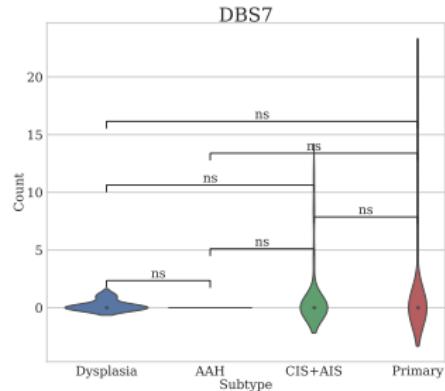
(a) Absolute



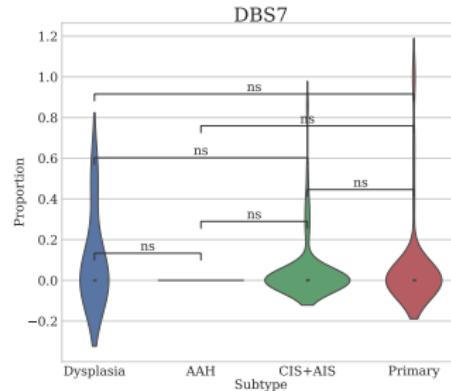
(b) Relative

Figure: DBS2 Signature in LUSC

DBS in LUAD IV



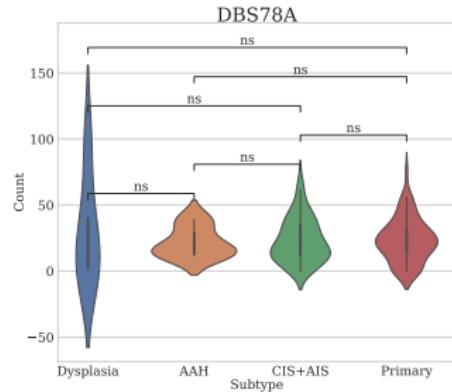
(a) Absolute



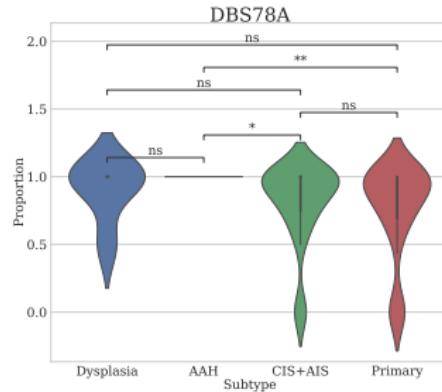
(b) Relative

Figure: DBS7 Signature in LUSC

DBS in LUAD V



(a) Absolute



(b) Relative

Figure: DBS78A Signature in LUSC

DBS in LUAD with Smoking I

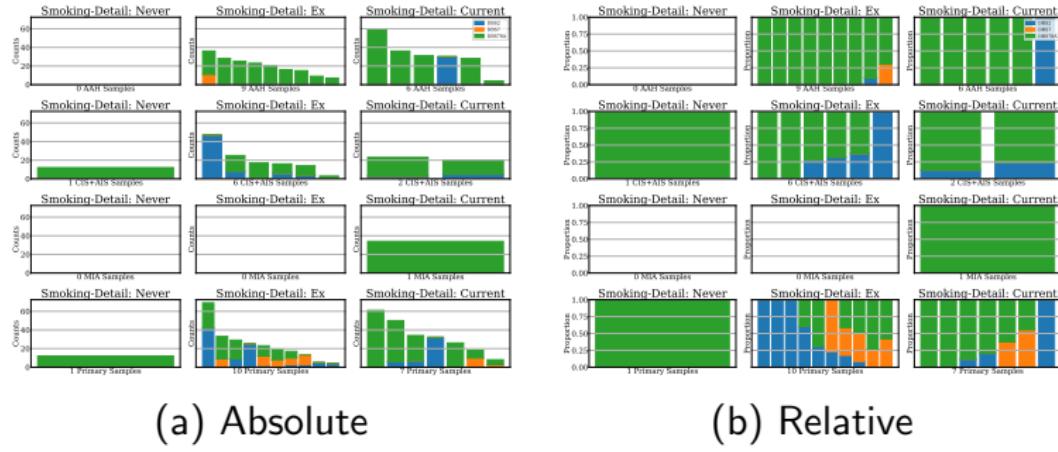
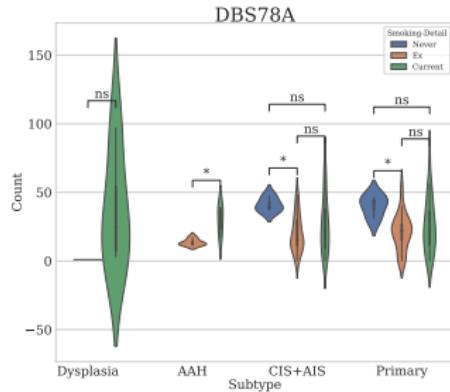
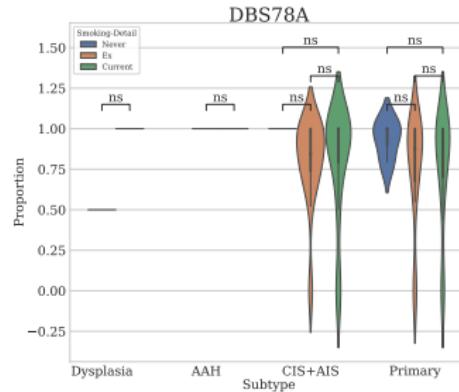


Figure: DBS Bar Plot by Cancer Subtype & Smoking in LUAD

DBS in LUAD with Smoking II



(a) Absolute



(b) Relative

Figure: DBS78A Signature in LUSC in Smoking

DBS in LUAD with Recurrence

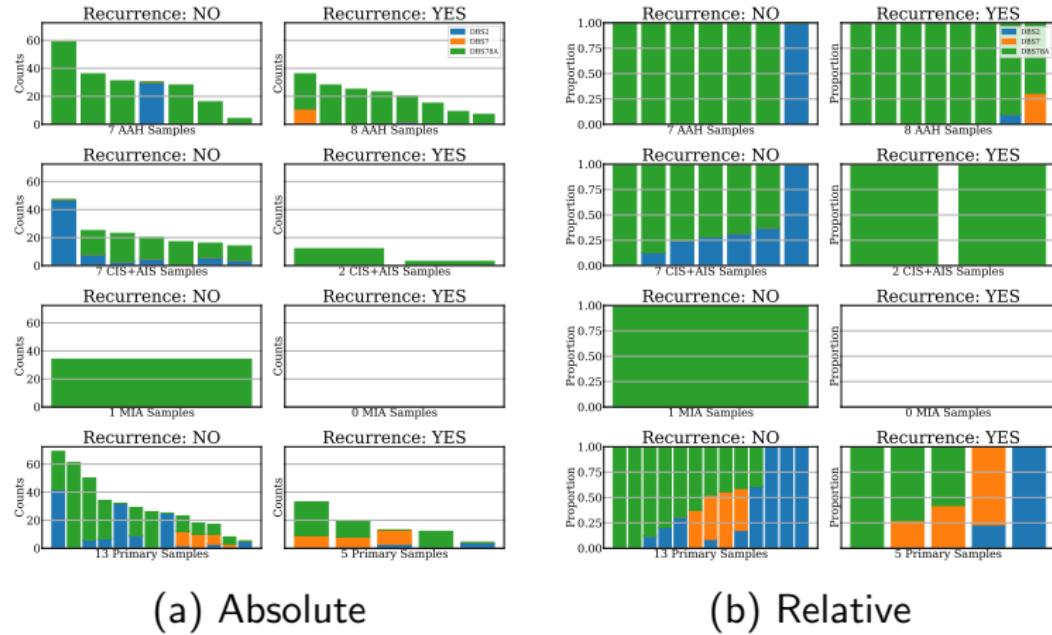


Figure: DBS Bar Plot by Cancer Subtype & Recurrence in LUAD

4. Results

4.8. Discovery of Mutational Signature

4.8.3. Short insertions & Deletions (Indels)

Indel signatures I

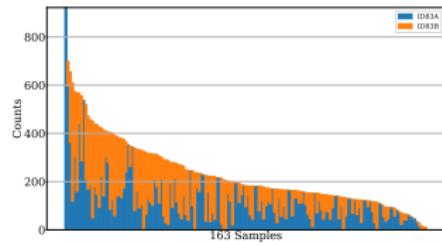
ID83A

content...

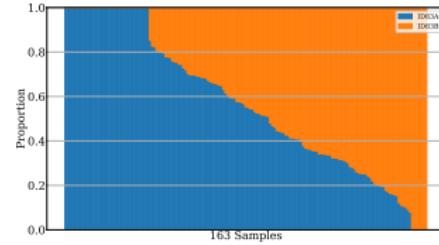
ID83B

content...

Indels in LUSC I



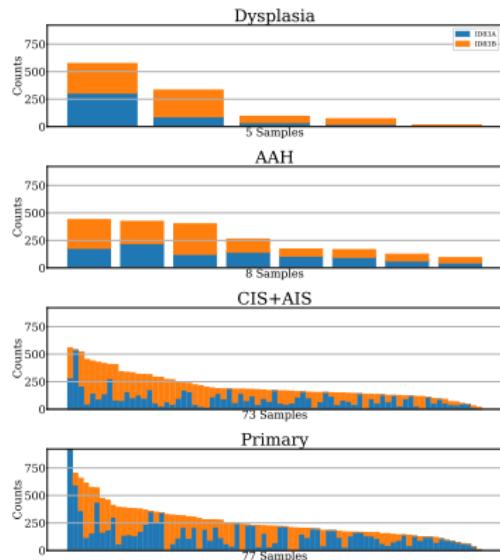
(a) Absolute



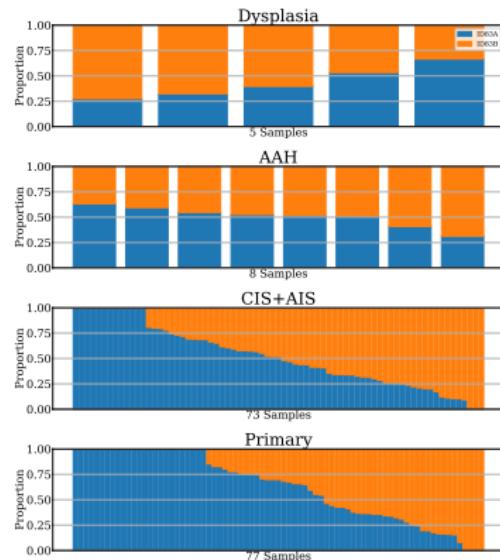
(b) Relative

Figure: Indel Bar Plot in LUSC

Indels in LUSC II



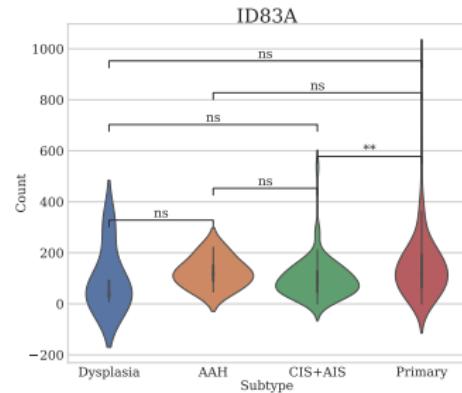
(a) Absolute



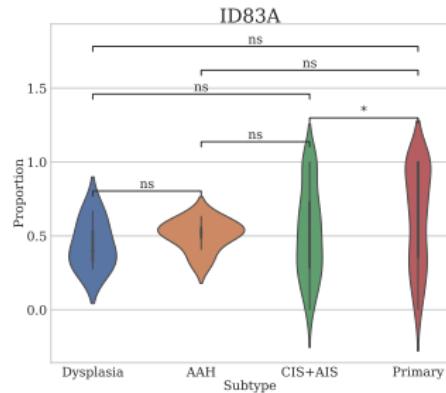
(b) Relative

Figure: Indel Bar Plot by Cancer Subtype in LUSC

Indels in LUSC III



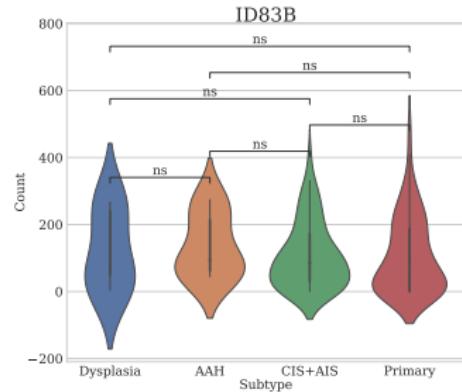
(a) Absolute



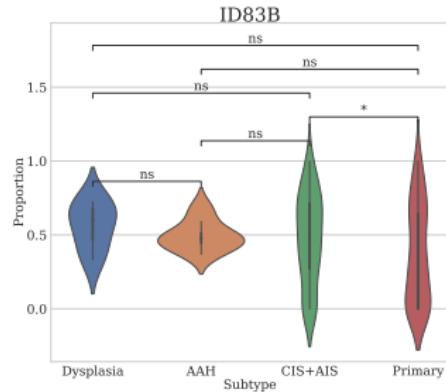
(b) Relative

Figure: Indel83A Signature in LUSC

Indels in LUSC IV



(a) Absolute



(b) Relative

Figure: Indel83B Signature in LUSC

Indel in LUSC with Smoking I

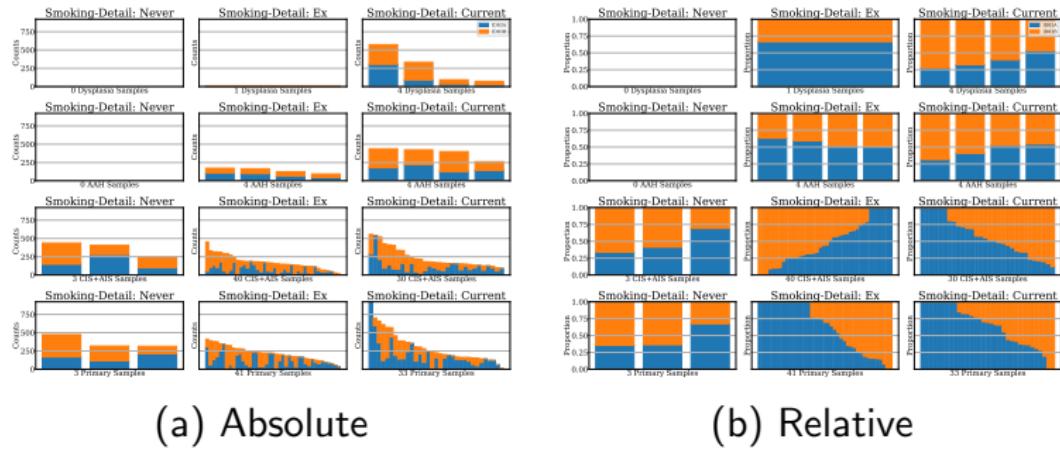
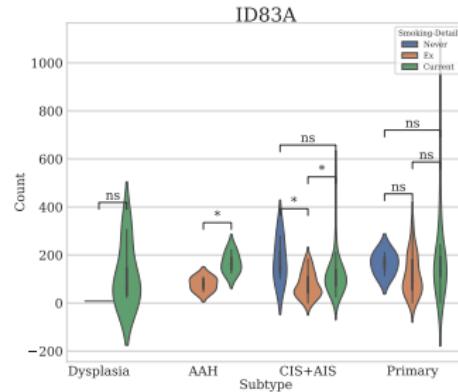
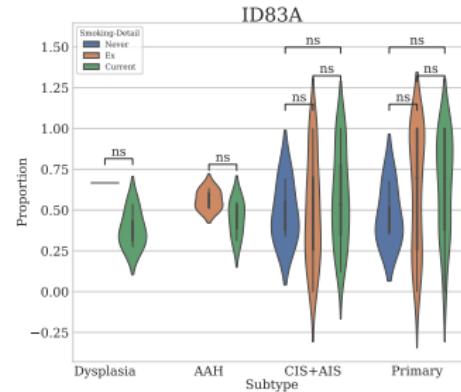


Figure: Indel Bar Plot by Cancer Subtype & Smoking in LUSC

Indel in LUSC with Smoking II



(a) Absolute



(b) Relative

Figure: Indel83A Signature in LUSC with Smoking

Indel in LUSC with Recurrence

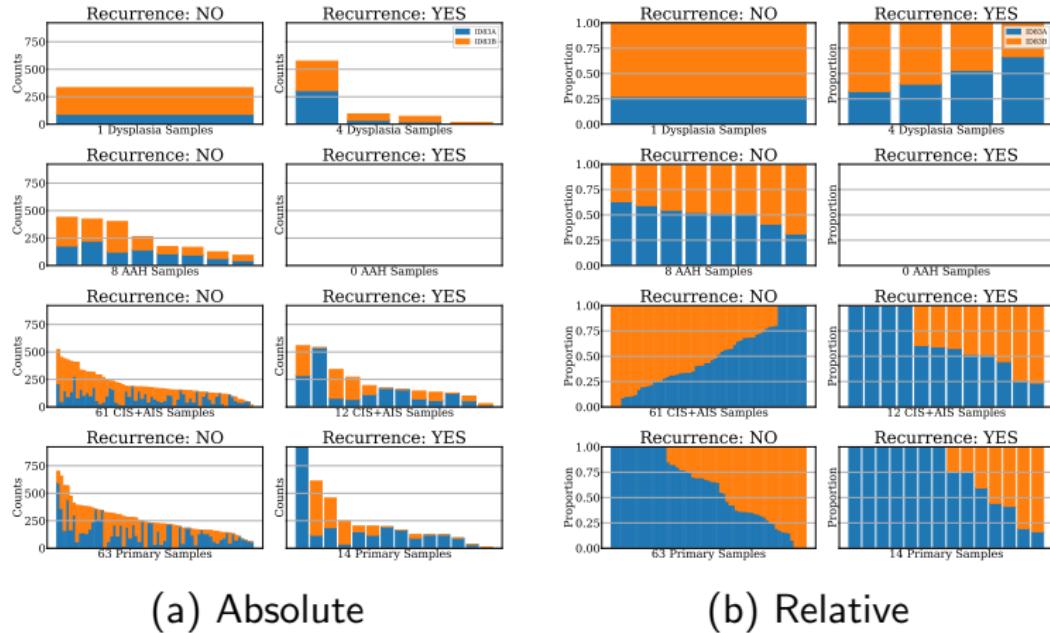
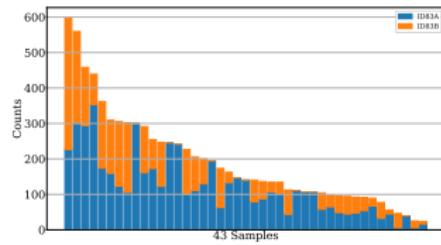
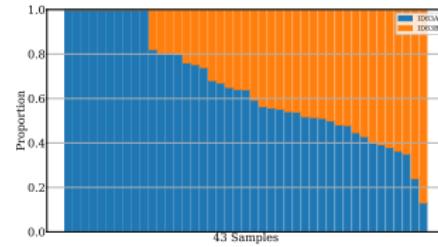


Figure: Indel Bar Plot by Cancer Subtype & Recurrence in LUSC

Indels in LUAD I



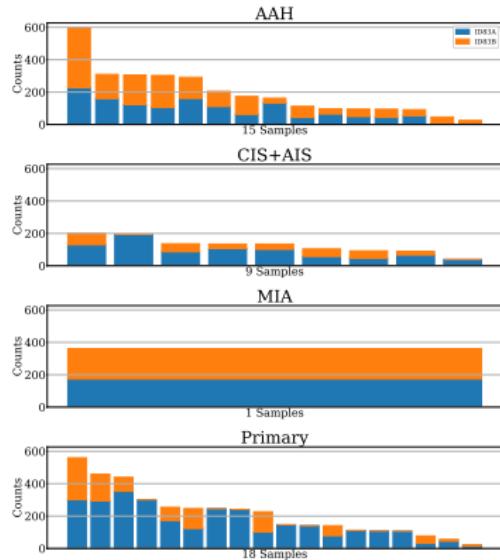
(a) Absolute



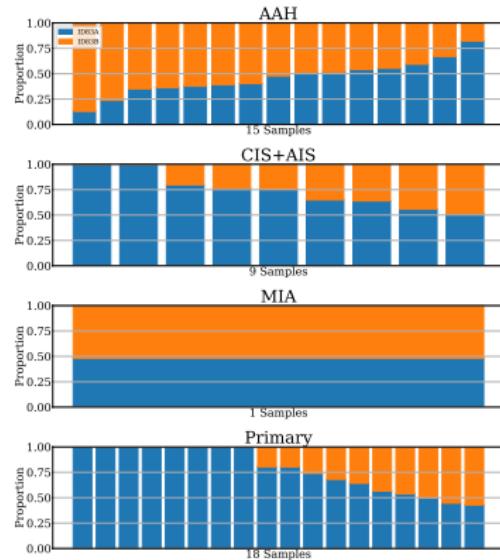
(b) Relative

Figure: Indel Bar Plot in LUAD

Indels in LUAD II



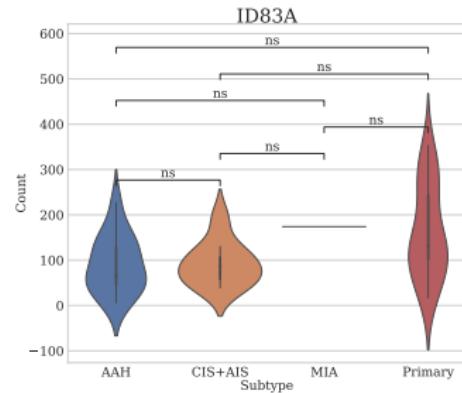
(a) Absolute



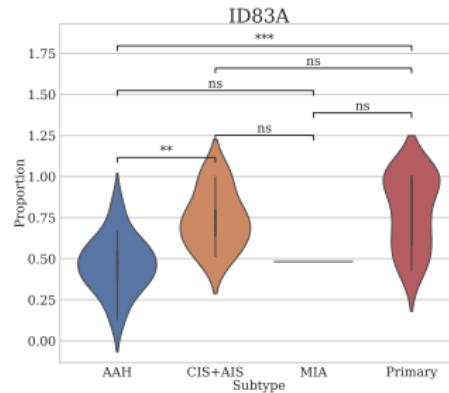
(b) Relative

Figure: Indel Bar Plot by Cancer Subtype in LUAD

Indels in LUAD III



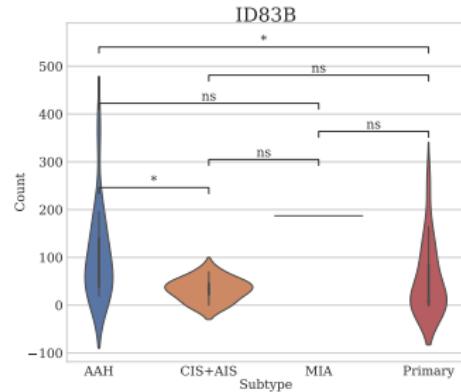
(a) Absolute



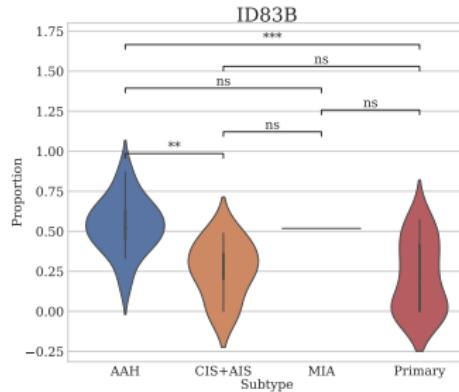
(b) Relative

Figure: Indel83A Signature in LUAD

Indels in LUAD IV



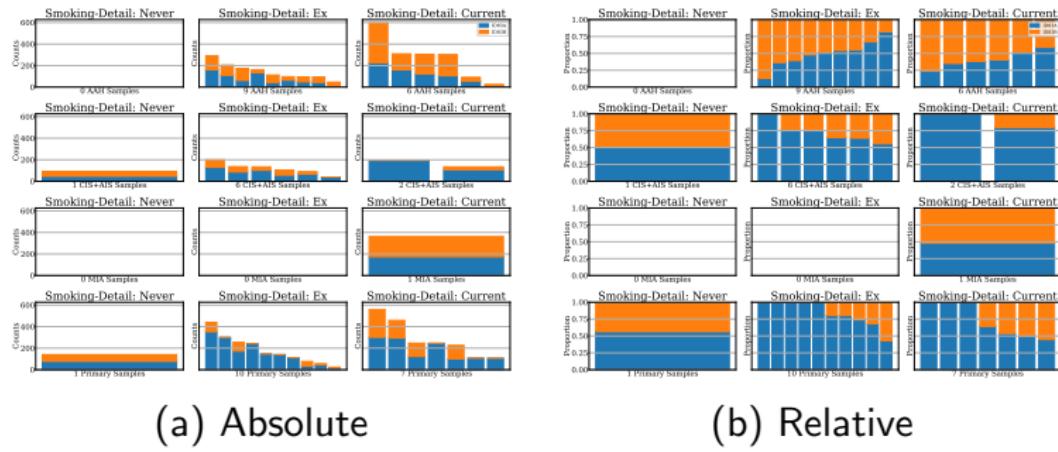
(a) Absolute



(b) Relative

Figure: Indel83B Signature in LUAD

Indel in LUAD with Smoking



Indel in LUAD with Recurrence

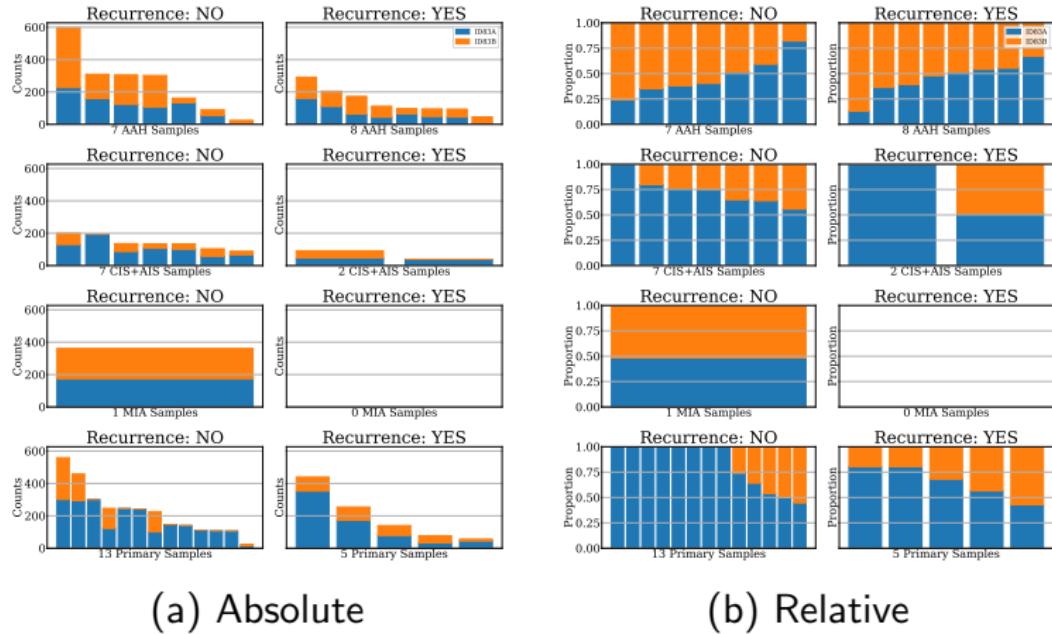


Figure: Indel Bar Plot by Cancer Subtype & Recurrence in LUAD

Findings in Mutation Signature

4. Results

4.9. Clinical Data with Point Mutation

Mutect2?

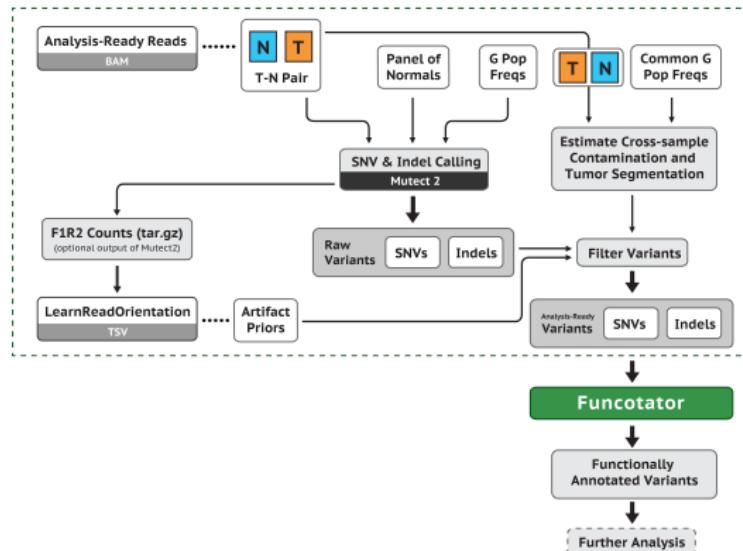


Figure: Somatic short variant discovery workflow (Van der Auwera et al., 2013; DePristo et al., 2011)

4. Results

4.9. Clinical Data with Point Mutation

4.9.1. For Smoking

LUSC with Smoking

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 | |

Clinical Data about LUSC for Smoking I

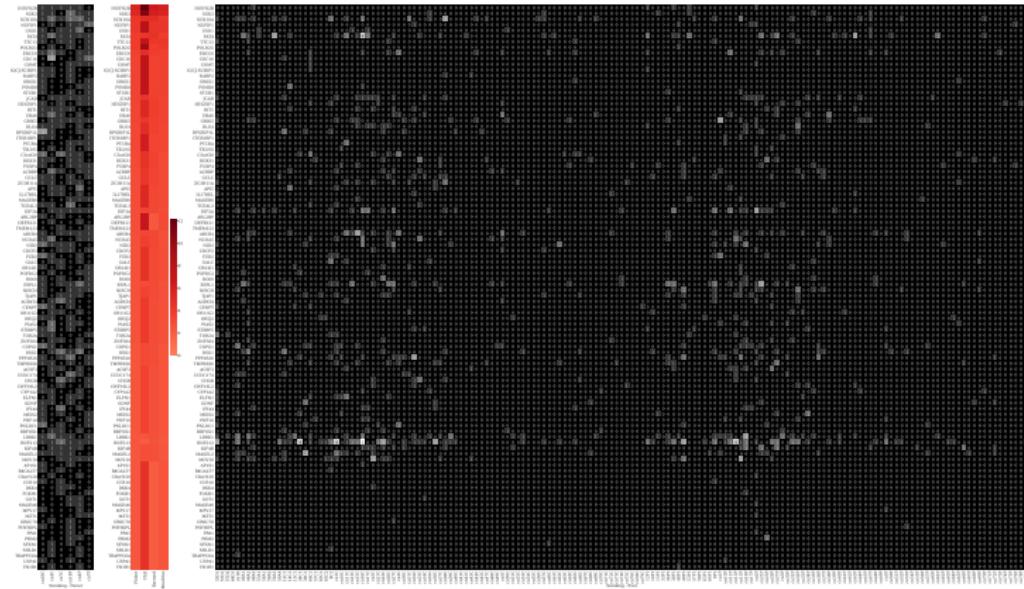


Figure: Clinical Data about LUSC for Smoking

Clinical Data about LUSC for Smoking II

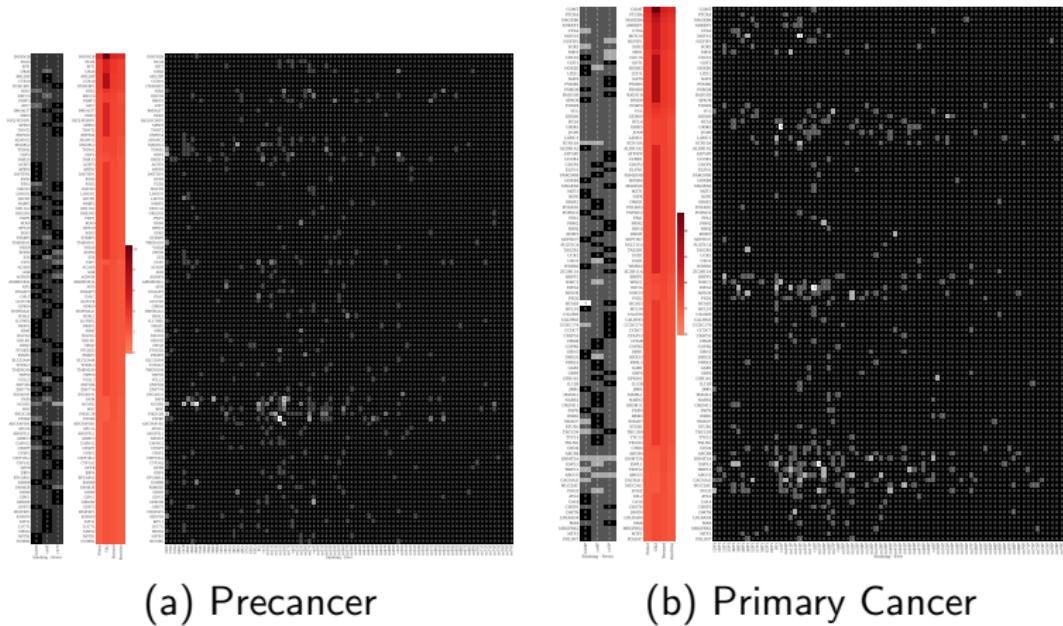


Figure: Clinical Data about LUSC for Smoking with Precancer/Primary

Notable genes in LUSC for Smoking I

INSYN2B

- ① INSYN2B is the best indicator for Smoking.
- ② INSYN2B is the best indicator for Smoking in Precancer.

COMT

- ① COMT is the best indicator for Smoking in Primary.
- ② COMT catalyzes the O-methylation, and inactivates of neurotransmitters and hormones (Dawling, Roodi, Mernaugh, Wang, & Parl, 2001; J. Chen et al., 2011).
- ③

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

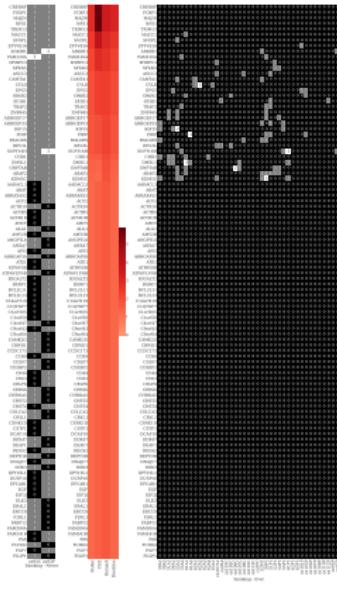
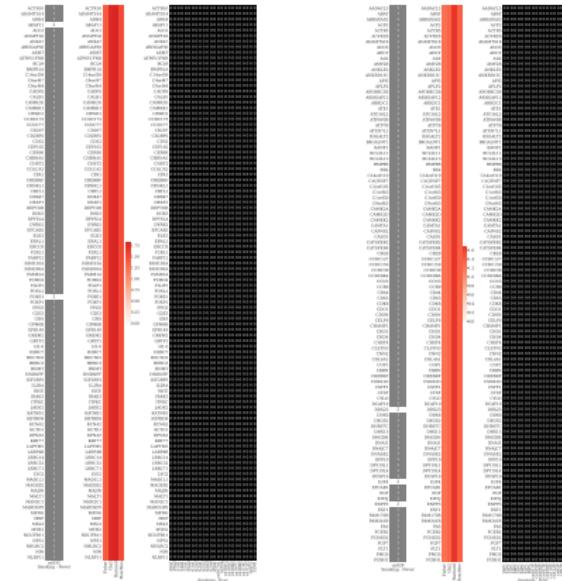


Figure: Clinical Data about LUAD for Smoking

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

- ① CREBRF is the best indicator for Smoking.

ACTR10

- ① ACTR10 is the best indicator for Smoking in Precancer.

AADACL3

- ① AADACL3 is the best indicator for Smoking in Primary.

4. Results

4.9. Clinical Data with Point Mutation

4.9.2. 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

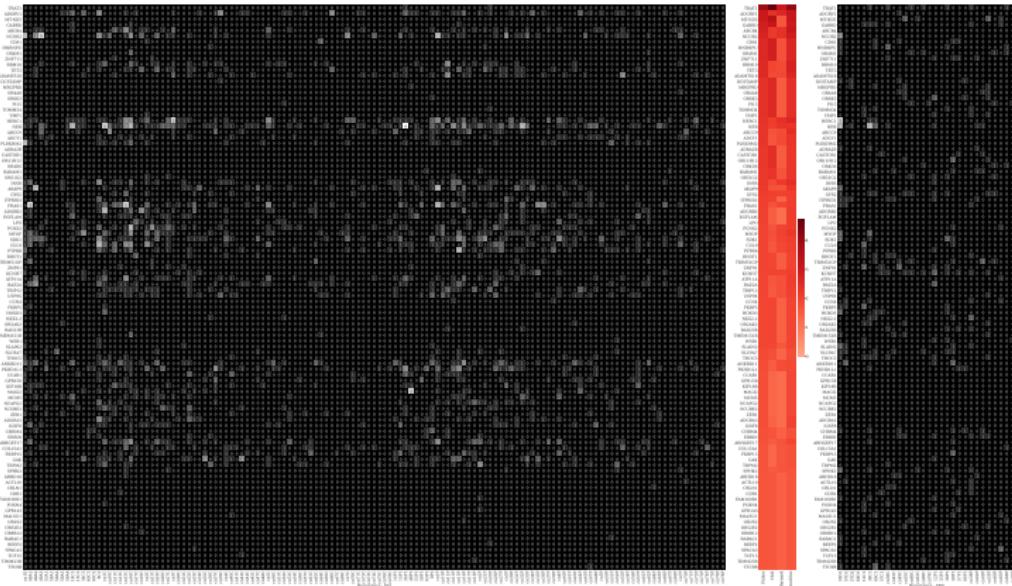


Figure: Clinical Data about LUSC for Recurrence

Clinical Data about LUSC for Recurrence II

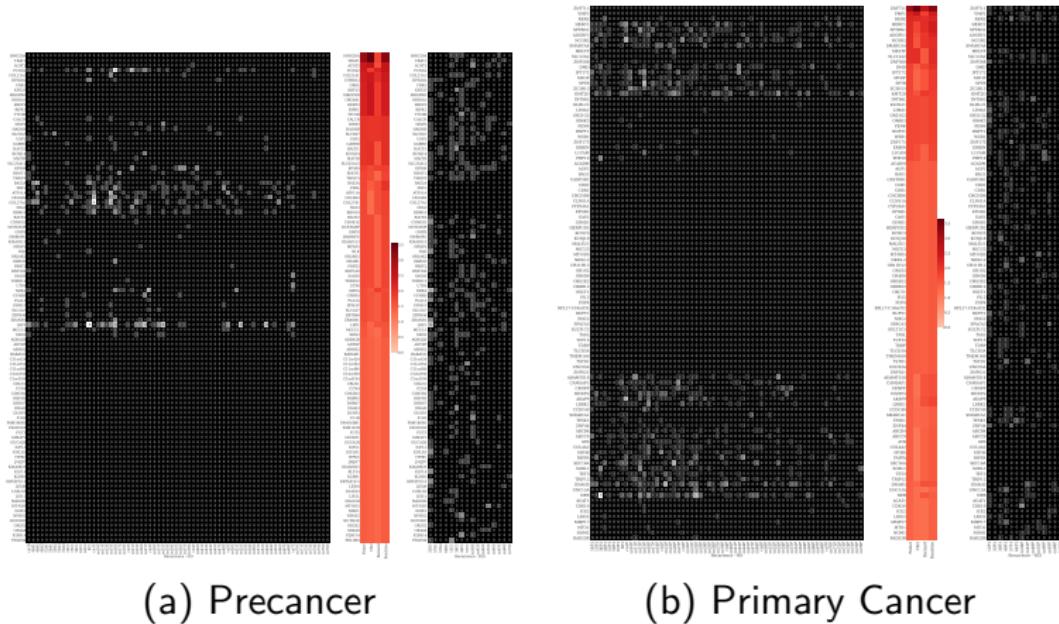


Figure: Clinical Data about LUSC for Recurrence with Precancer/Primary

Notable genes in LUSC with Recurrence I

TRAT1

- ① TRAT1 is the best indicator for Recurrence.

HMG20A

- ① HMG20A is the best indicator for Recurrence in Precancer.

ZNF711

- ① ZNF711 is the best indicator for Recurrence in Primary.

LUAD with Recurrence

Table: LUAD WES Data with Recurrence

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

Clinical Data about LUAD for Recurrence I

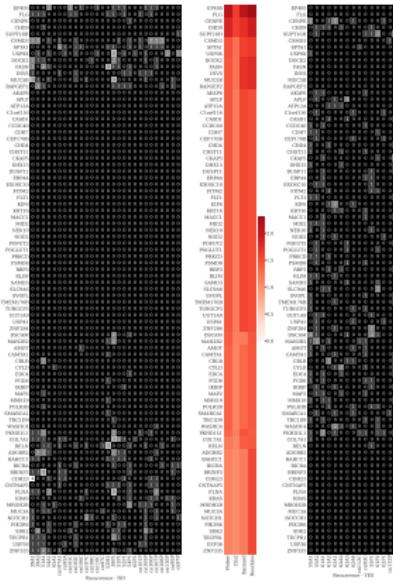
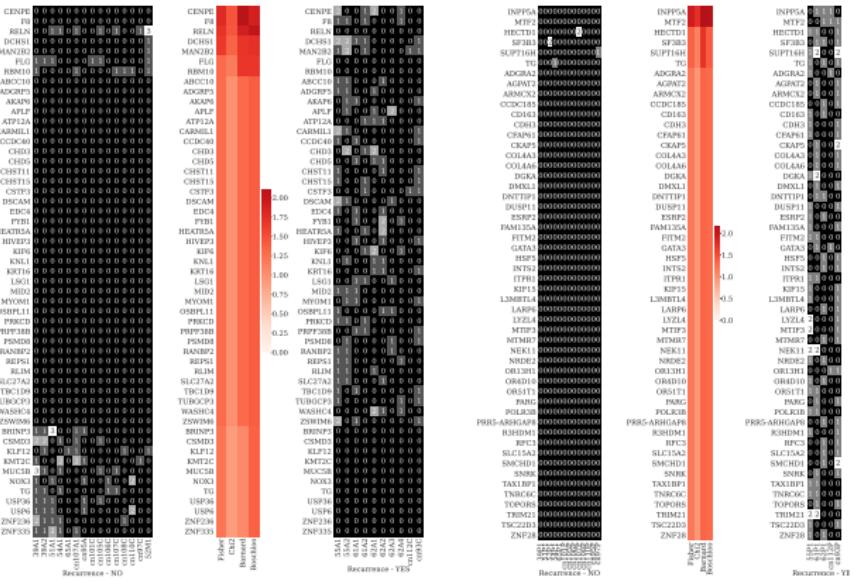


Figure: Clinical Data about LUAD for Recurrence

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

- ① EP400 is the best indicator for Recurrence.

CENPE

- ① CENPE is the best indicator for Recurrence in Precancer.

INPP5A

- ① INPP5A is the best indicator for Recurrence in Primary.

Findings in Clinical Data with Point Mutations

4. Results

4.10. Differences in Gene Expression Levels

RSEM?

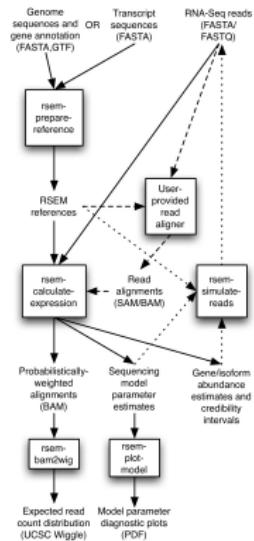


Figure: RSEM workflow (B. Li & Dewey, 2011)

DESeq2?

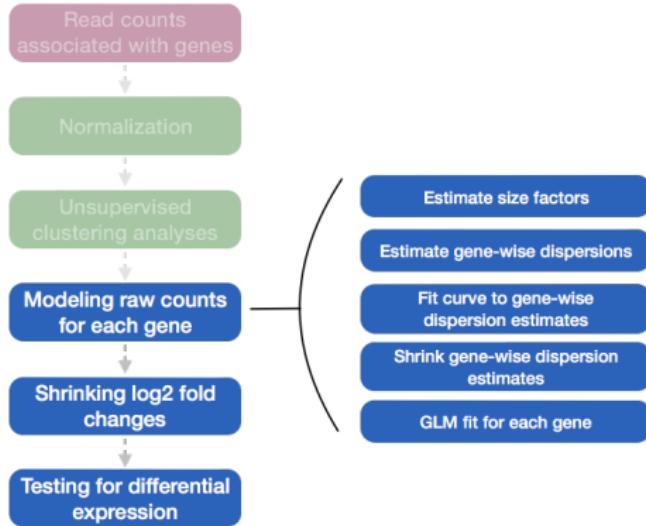


Figure: DESeq2 workflow (Love, Huber, & Anders, 2014)

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?

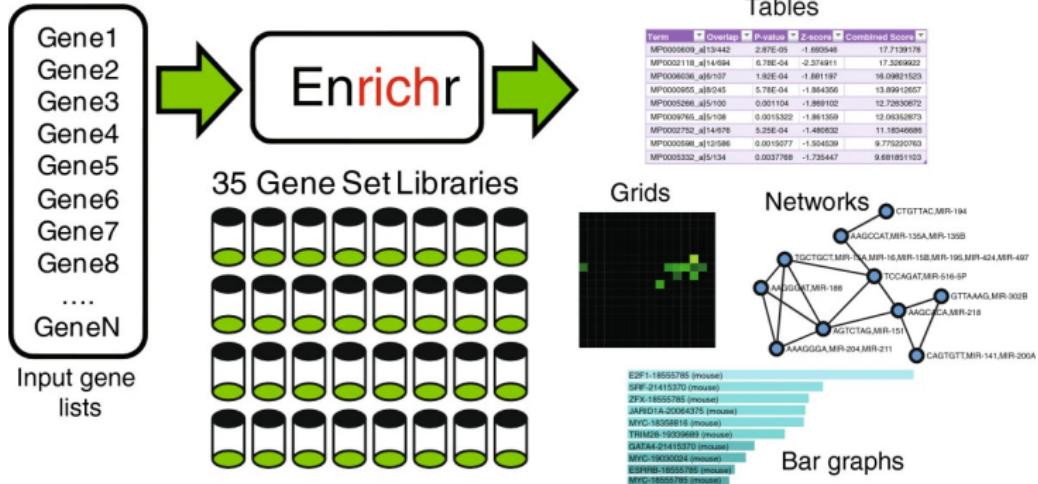


Figure: Enrichr workflow (E. Y. Chen et al., 2013; Kuleshov et al., 2016)

Gene-set Library

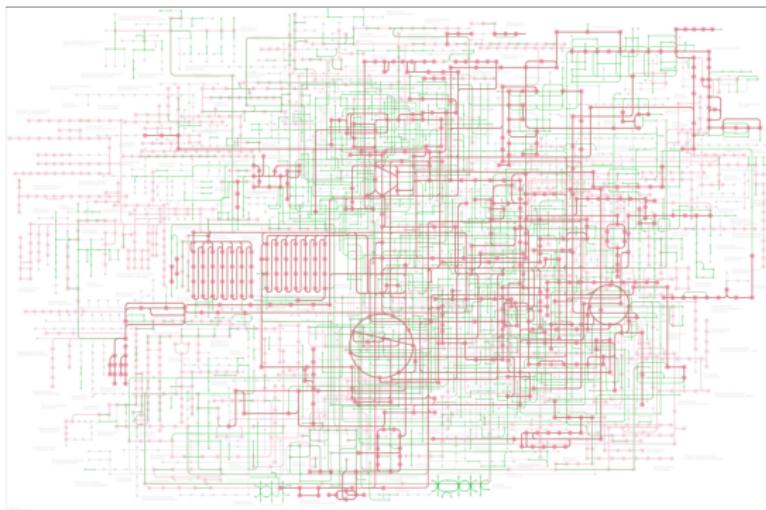


Figure: The global map of metabolic pathways by KEGG (Kanehisa et al., 2021)

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 | |

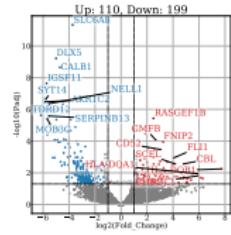
4. Results

4.10. Differences in Gene Expression Levels

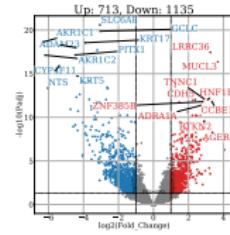
4.10.1. Comparing cancer stage in LUSC

DEG Volcano Plots in LUSC

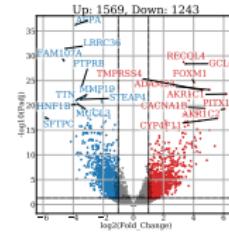
Normal → Dysplasia → CIS → Primary (LUSC)



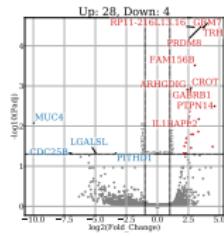
(a) Normal-Dysplasia



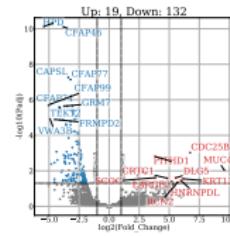
(b) Normal-CIS



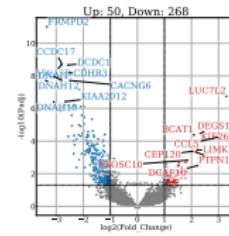
(c) Normal-Primary



(d) Dysplasia-CIS



(e) Dysplasia-Primary

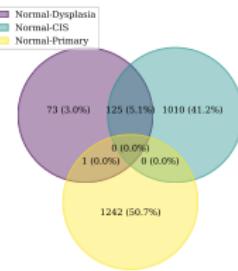
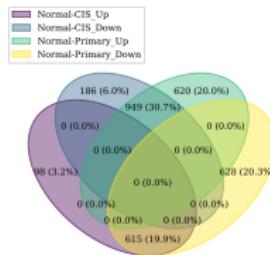
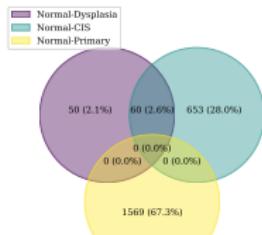


(f) CIS-Primary

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

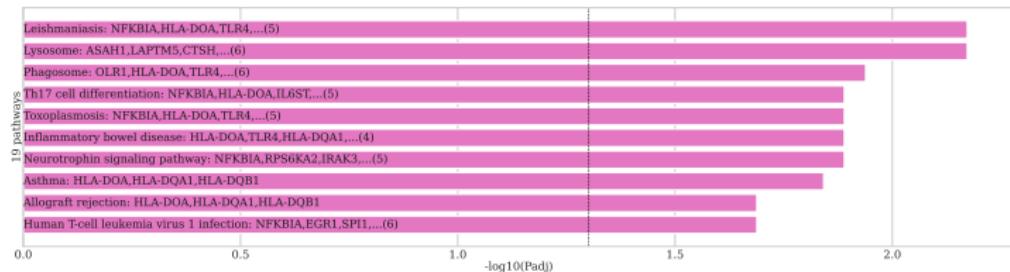


Figure: Up-regulated Pathways on Normal vs. Dysplasia



Figure: Down-regulated Pathways on Normal vs. Dysplasia

Enrichment test with Normal vs. CIS in LUSC

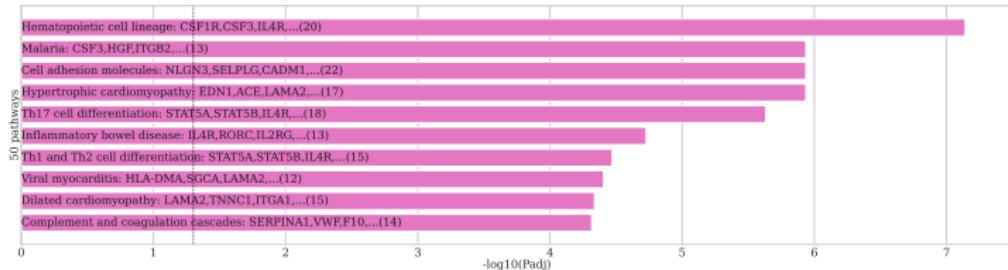


Figure: Up-regulated Pathways on Normal vs. CIS

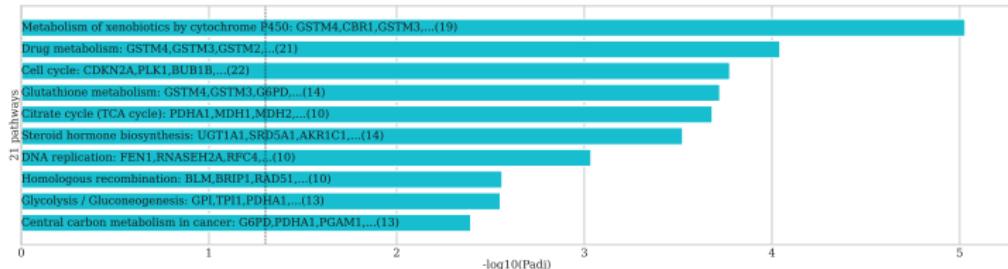


Figure: Down-regulated Pathways on Normal vs. CIS

Enrichment test with Normal vs. Primary in LUSC

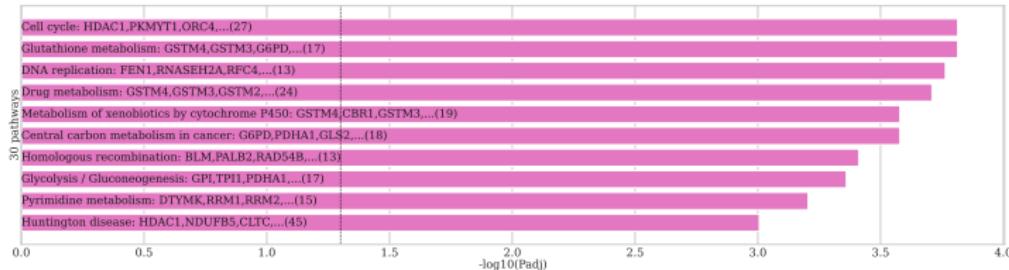


Figure: Up-regulated Pathways on Normal vs. Primary

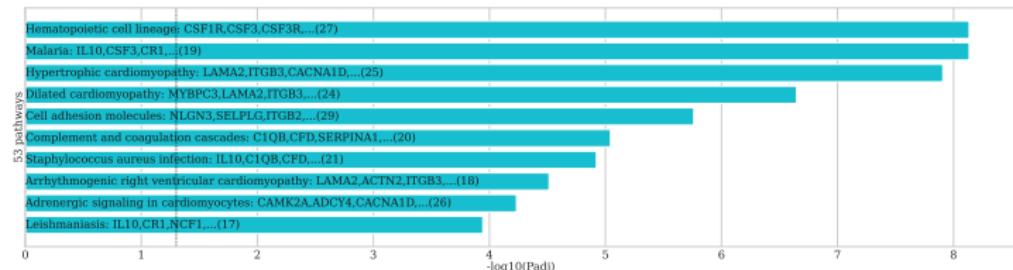


Figure: Down-regulated Pathways on Normal vs. Primary

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 (Z. Lin et al., 2018).
- ③ SFTPC $\downarrow \Rightarrow$ Poor survival in LUAD (B. Li et al., 2019).
- ④ Associated with lung disease in adult (Henderson et al., 2013) and baby (Brasch et al., 2004).

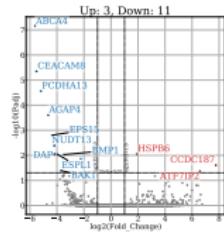
4. Results

4.10. Differences in Gene Expression Levels

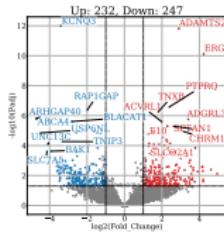
4.10.2. Comparing cancer stage in LUAD

DEG Volcano Plots in LUAD

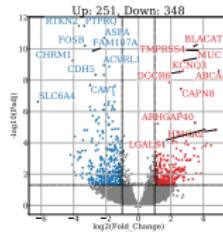
Normal → AAH → AIS → Primary (LUAD)



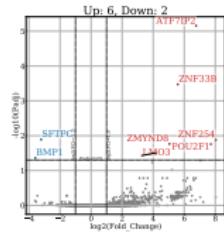
(a) Normal-AAH



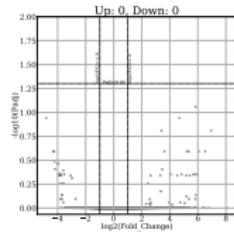
(b) Normal-AIS



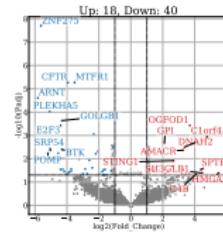
(c) Normal-Primary



(d) AAH-AIS



(e) AAH-Primary

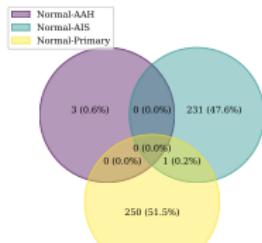


(f) AIS-Primary

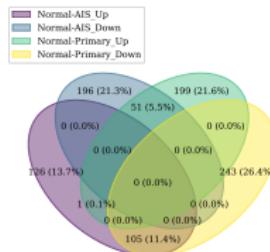
Figure: DEG Volcano Plots in LUAD

DEG Venn Diagram in LUAD

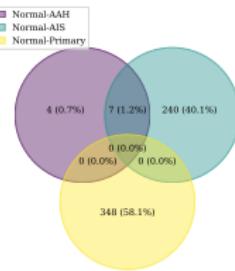
Normal → AAH → AIS → Primary (LUAD)



(a) Up-regulated



(b) Both



(c) Down-regulated

Figure: DEG Venn Diagram in LUAD

Enrichment test with Normal vs. AAH in LUAD

Nothing to show...

Figure: Up-regulated Pathways on Normal vs. AAH

Nothing to show...

Figure: Down-regulated Pathways on Normal vs. AAH

Enrichment test with Normal vs. AIS in LUAD

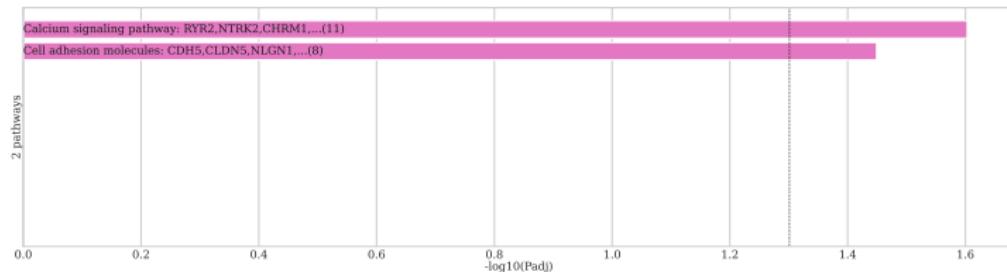


Figure: Up-regulated Pathways on Normal vs. AIS



Figure: Down-regulated Pathways on Normal vs. AIS

Enrichment test with Normal vs. Primary in LUAD

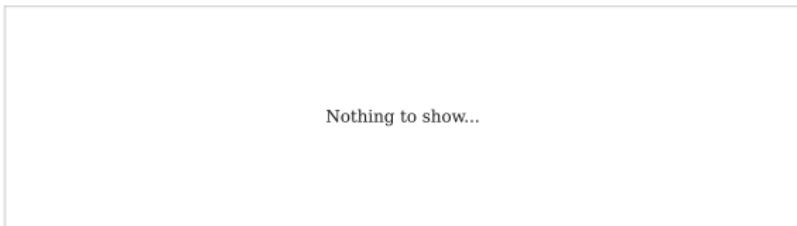


Figure: Up-regulated Pathways on Normal vs. Primary

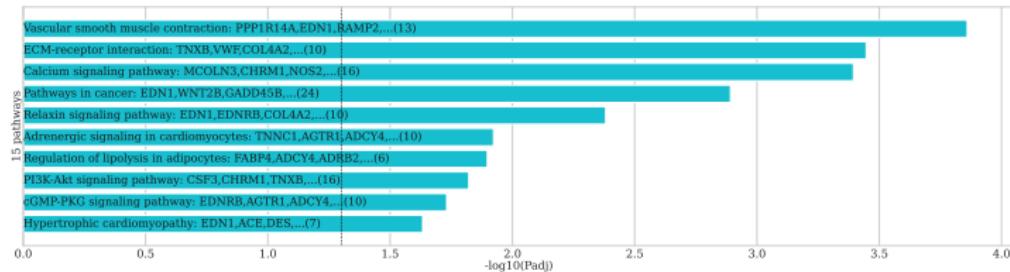


Figure: Down-regulated Pathways on Normal vs. Primary

ABCA4

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

Finding in Comparing cancer stage in LUAD II

KCNQ3

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

CHRM1

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

4. Results

4.10. Differences in Gene Expression Levels

4.10.3. Recur vs. Non-recur in LUSC

Table: Number of WTS LUSC samples

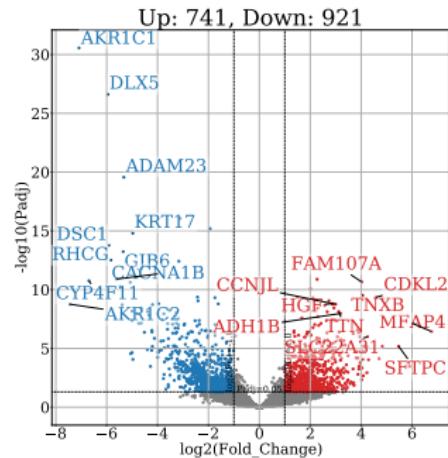
| Recurrence? | Stage | Number of Samples | |
|----------------|-----------|-------------------|-----------|
| | | Normal | Dysplasia |
| Recurrence | Normal | 1 | |
| | Dysplasia | | 1 |
| | CIS+AIS | 5 | |
| | Primary | 6 | |
| | Total | 13 | |
| Non-recurrence | Normal | 16 | |
| | Dysplasia | | 1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

Pooled normal samples

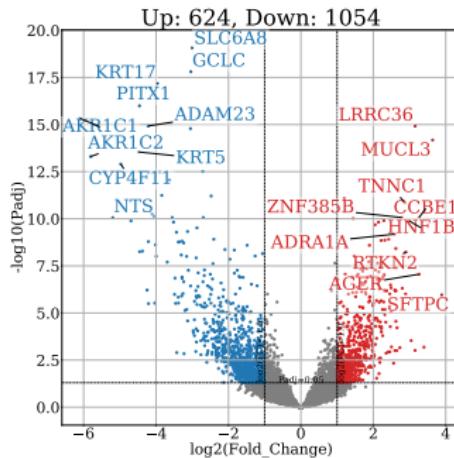
In order to compare with Normal stage, merging Normal samples.

∴ 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

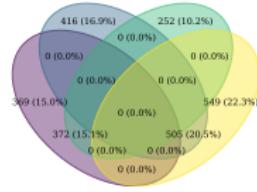
DEG Venn Diagram for R vs. NR with CIS in LUSC

Recur
Non-recur



(a) Up-regulated

Recur_Up
Recur_Down
Non-recur_Up
Non-recur_Down



(b) Both

Recur
Non-recur



(c) Down-regulated

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

Enrichment test for Recur-specific with CIS in LUSC

Nothing to show...

Figure: Up-regulated Pathways for Recur-specific

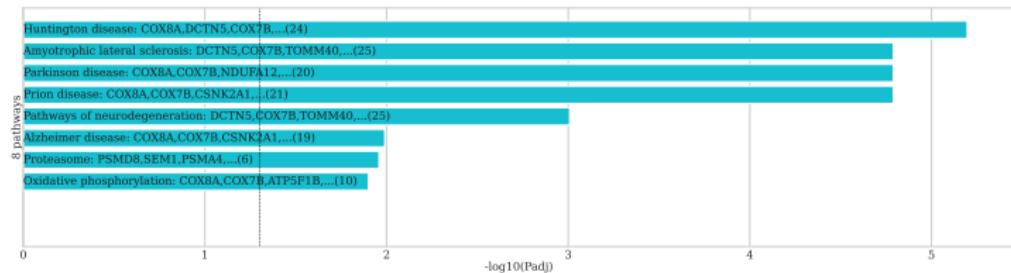


Figure: Down-regulated Pathways for Recur-specific

Enrichment test for Non-recr-specific with CIS in LUSC

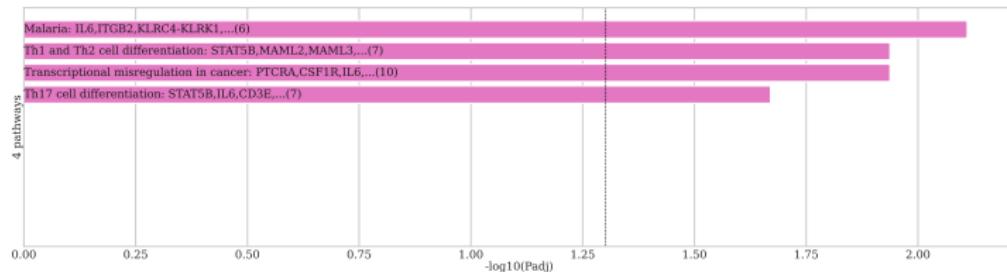


Figure: Up-regulated Pathways for Non-recr-specific

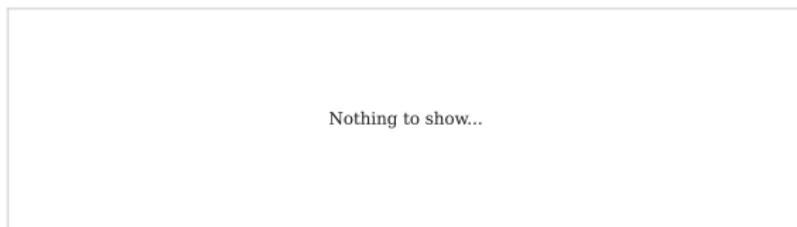


Figure: Down-regulated Pathways for Non-recr-specific

Enrichment test for Intersected with CIS in LUSC

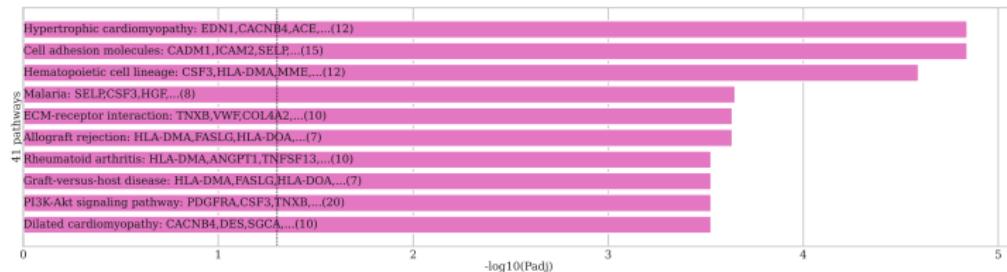


Figure: Up-regulated Pathways for Intersected

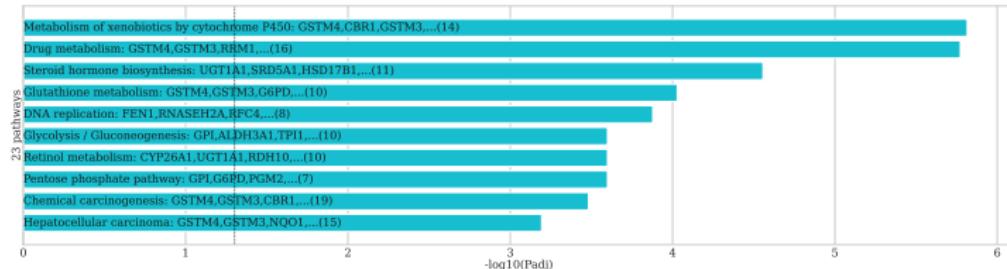
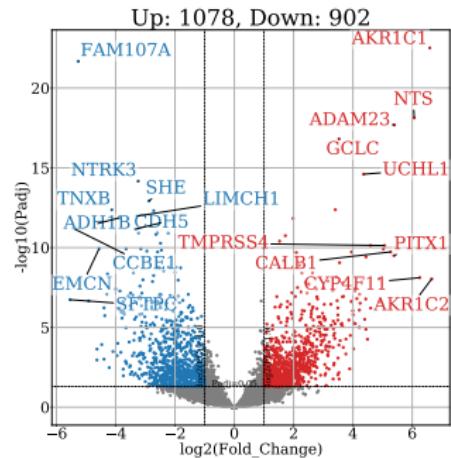
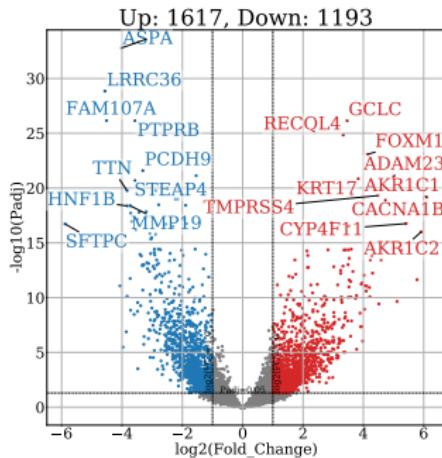


Figure: Down-regulated Pathways for Intersected

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



(a) Recur

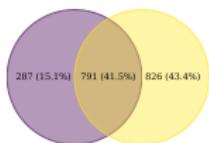


(b) Non-recur

Figure: DEG Volcanot Plot with Primary in LUSC

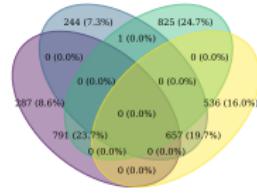
DEG Venn Diagram for R vs. NR with Primary in LUSC

Recur
Non-recur



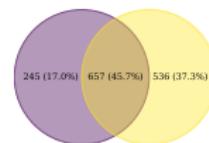
(a) Up-regulated

Recur_Up
Recur_Down
Non-recur_Up
Non-recur_Down



(b) Both

Recur
Non-recur



(c) Down-regulated

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

Enrichment test for Recur-specific with Primary in LUSC

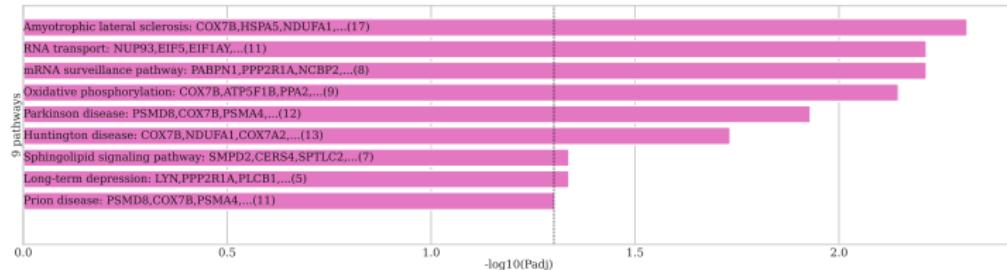


Figure: Up-regulated Pathways for Recur-specific



Figure: Down-regulated Pathways for Recur-specific

Enrichment test for NR-specific with Primary in LUSC

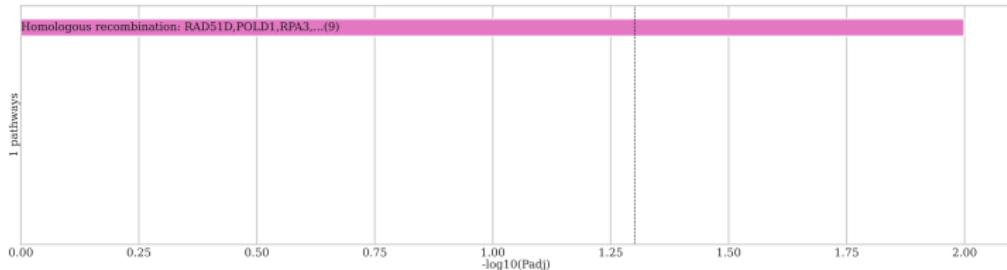


Figure: Up-regulated Pathways for Non-recur-specific

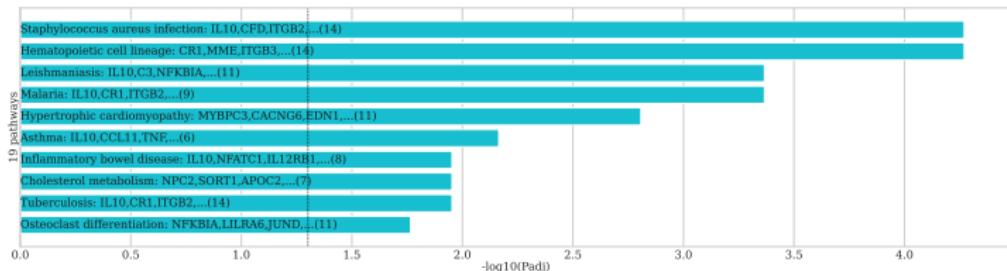


Figure: Down-regulated Pathways for Non-recur-specific

Enrichment test for Intersected with Primary in LUSC

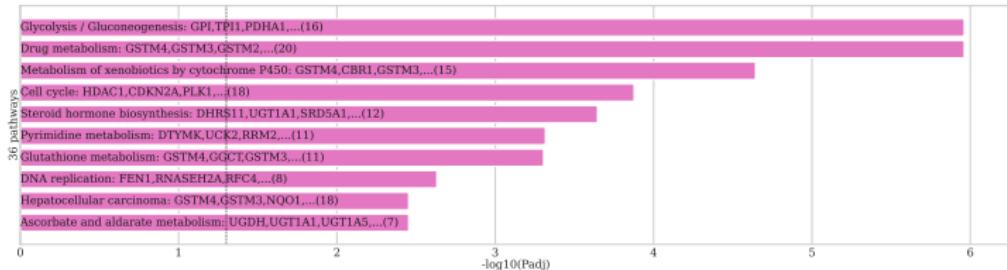


Figure: Up-regulated Pathways for Intersected

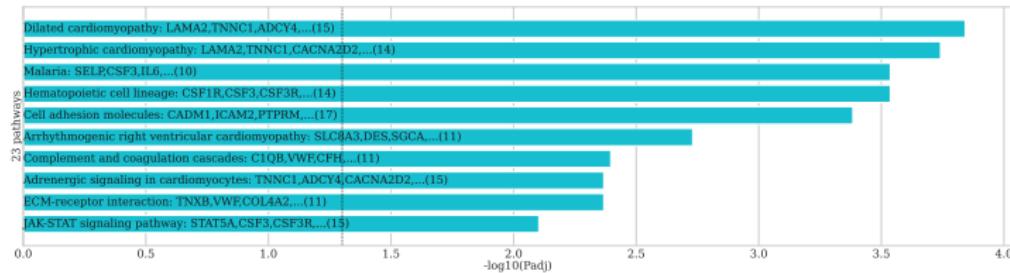


Figure: Down-regulated Pathways for Intersected

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

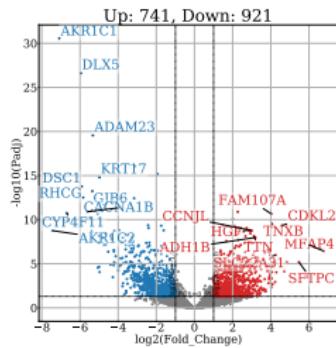
- ① Highly up-regulated in Non-recur patients.
- ② DNA-dependent ATPase (Yin, Kwon, Varshavsky, & Wang, 2004)
- ③ RECQL4 modulate chromosome segregation (Yin et al., 2004)
- ④ RECQL5 promotes metastasis & resistance in NSCLC (Xia, Zhang, Yuan, & Niu, 2021)
- ⑤ RECQL4 ↑ ⇒ Poor prognosis in breast cancer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

4. Results

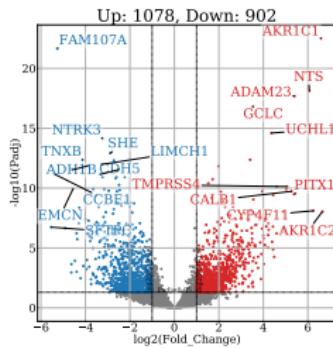
4.10. Differences in Gene Expression Levels

4.10.4. Within Recur in LUSC

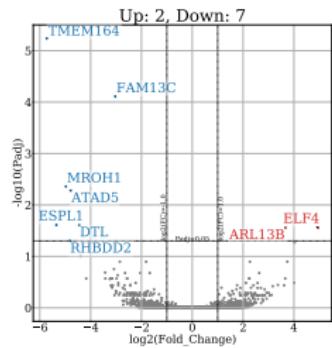
DEG Volcano Plots with Recur in LUSC



(a) Normal-CIS



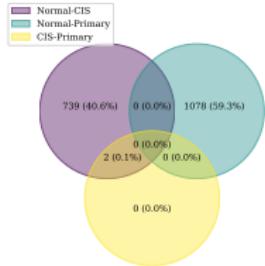
(b) Normal-Primary



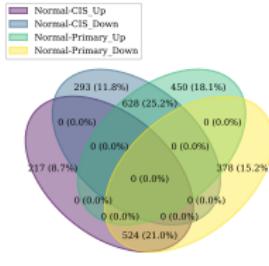
(c) CIS-Primary

Figure: DEG Volcano Plots with Recur samples in LUSC

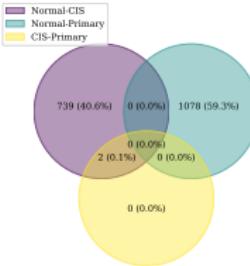
DEG Venn Diagram with Recur in LUSC



(a) Up-regulated



(b) Both



(c) Down-regulated

Figure: DEG Venn Diagram with Recur samples in LUSC

Enrichment test with Normal vs. CIS for Recur

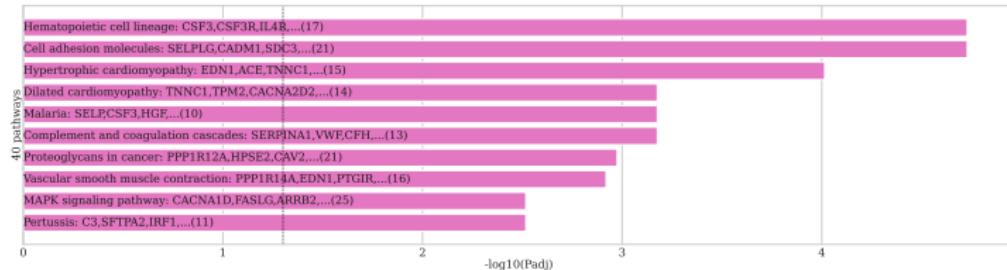


Figure: Up-regulated Pathways on Normal vs. CIS for Recur in LUSC

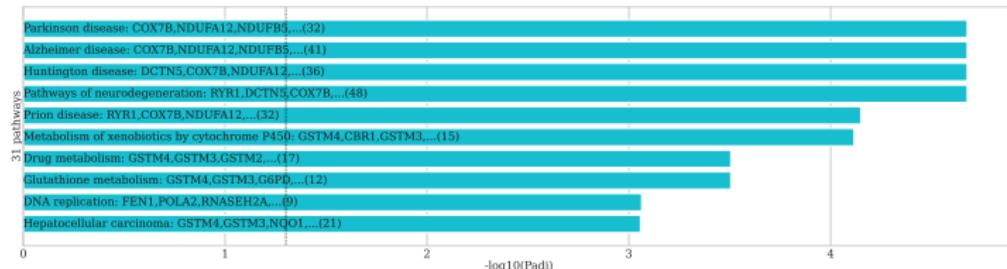


Figure: Down-regulated Pathways on Normal vs. CIS for Recur in LUSC

Enrichment test with Normal vs. Primary for Recur

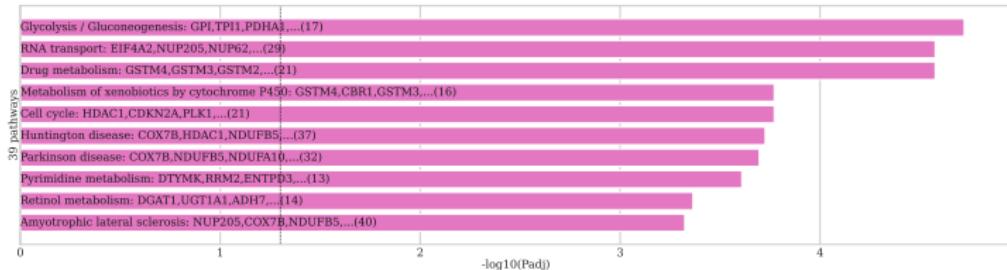


Figure: Up-regulated Pathways on Normal vs. Primary for Recur in LUSC

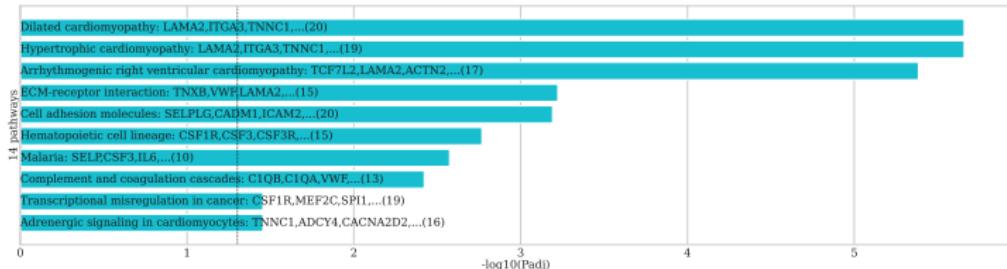


Figure: Down-regulated Pathways on Normal vs. Primary for Recur in LUSC

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

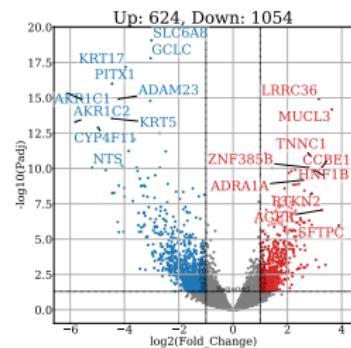
- ① Up-regulated in CIS, but down-regulated in Primary.
- ② May play a role in tumor development (L. Wang et al., 2000)
- ③ Negatively regulates focal adhesion assembly (Le et al., 2010)

4. Results

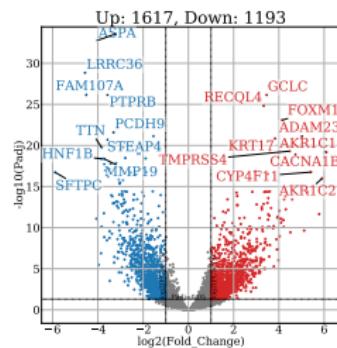
4.10. Differences in Gene Expression Levels

4.10.5. Within Non-recur in LUSC

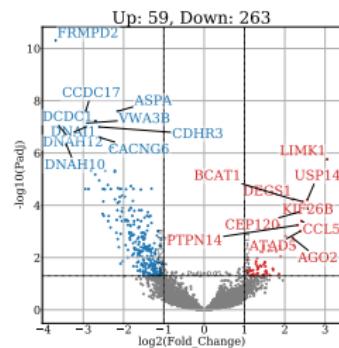
DEG Volcano Plots with Non-recur in LUSC



(a) Normal-CIS



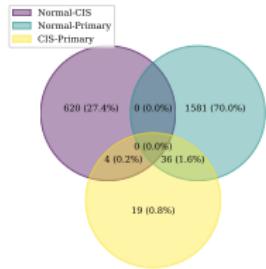
(b) Normal-Primary



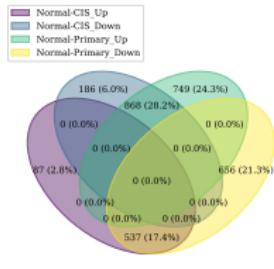
(c) CIS-Primary

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

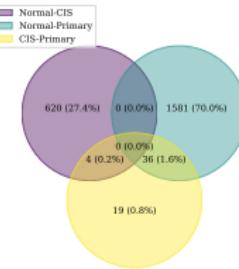
DEG Venn Diagram with Non-recur in LUSC



(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

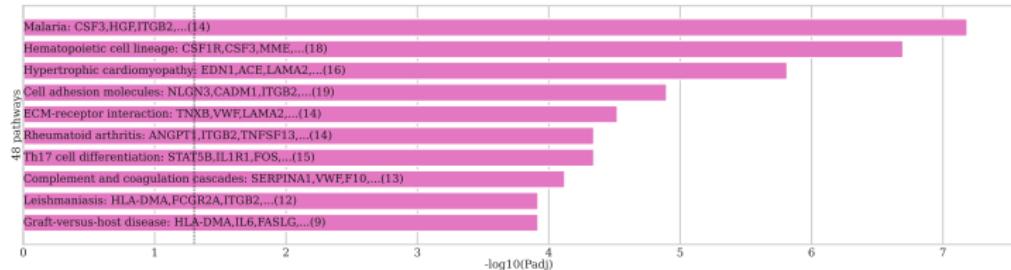


Figure: Up-regulated Pathways on Normal vs. CIS for Non-recur in LUSC

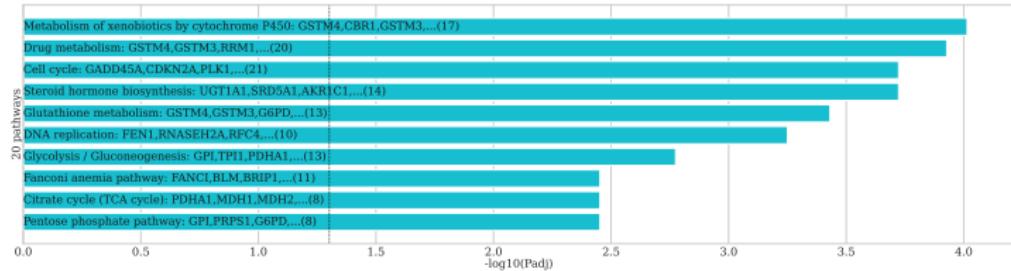


Figure: Down-regulated Pathways on Normal vs. CIS for Non-recur in LUSC

Enrichment test with Normal vs. Primary for Non-recur

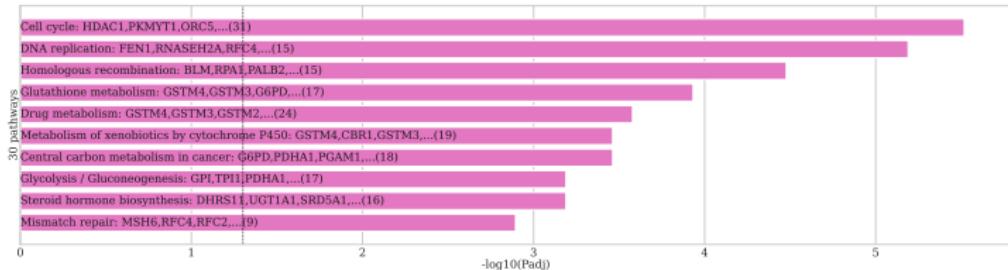


Figure: Up-regulated Pathways on Normal vs. Primary for Non-recur in LUSC

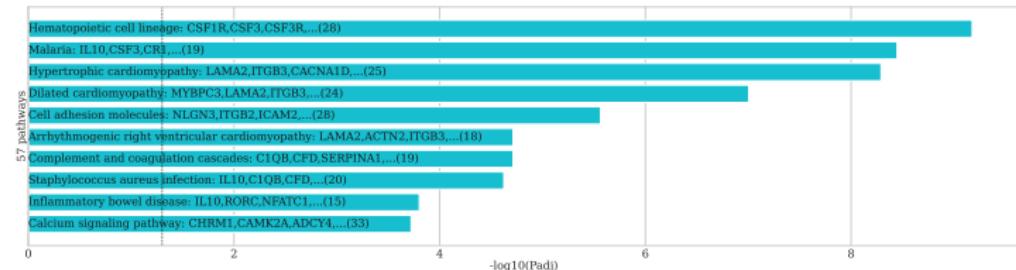


Figure: Down-regulated Pathways on Normal vs. Primary for Non-recur in LUSC

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

4.10. Differences in Gene Expression Levels

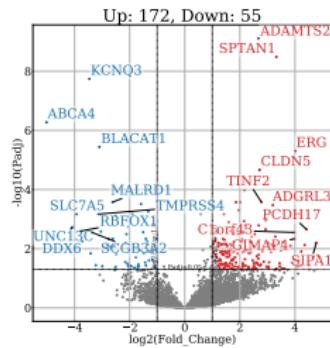
4.10.6. Within Non-recur in LUAD

LUAD Data Composition

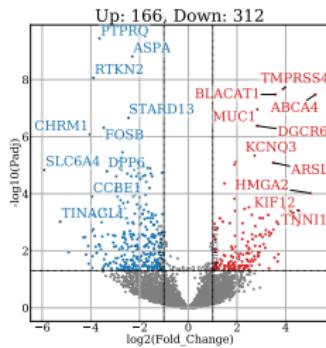
Table: Number of WTS LUAD samples

| 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 |

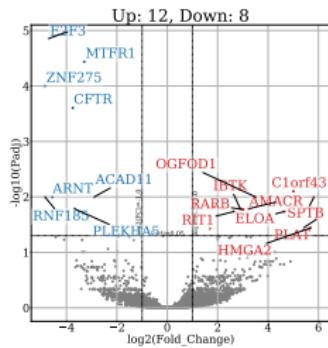
DEG Volcano Plots with Non-recr in LUAD



(a) Normal-AIS



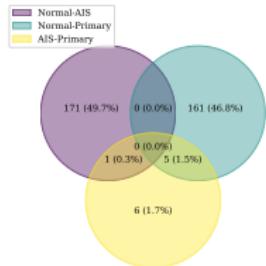
(b) Normal-Primary



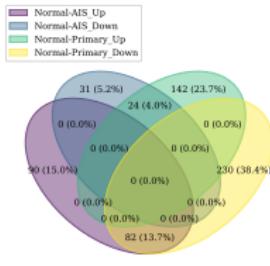
(c) AIS-Primary

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

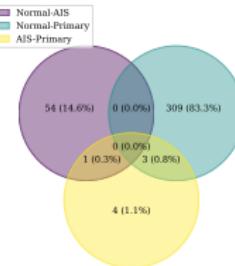
DEG Venn Diagram with Non-recur in LUAD



(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

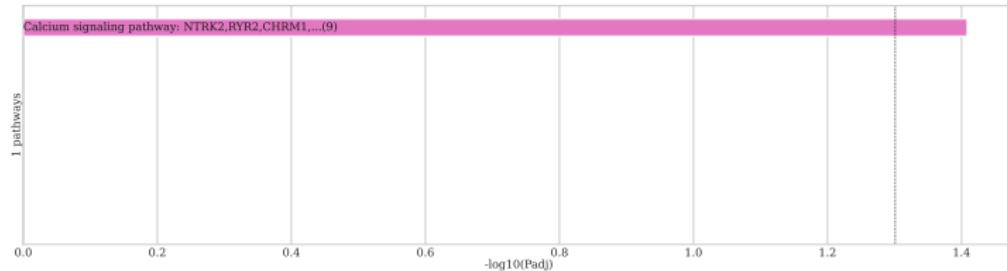


Figure: Up-regulated Pathways on Normal vs. AIS for Non-recur in LUAD

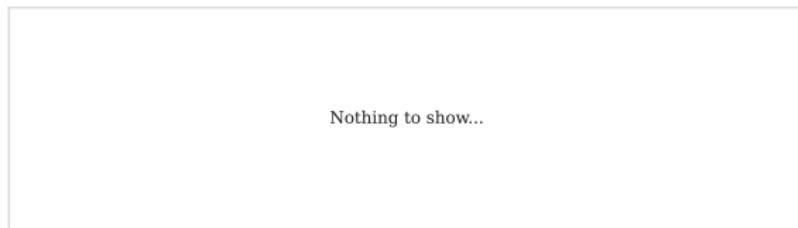


Figure: Down-regulated Pathways on Normal vs. AIS for Non-recur in LUAD

Enrichment test with Normal vs. Primary in LUAD

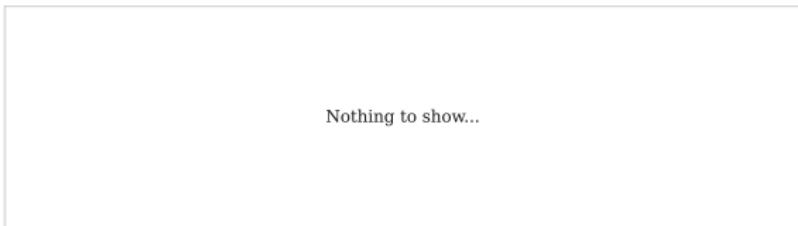


Figure: Up-regulated Pathways on Normal vs. Primary for Non-recur in LUAD

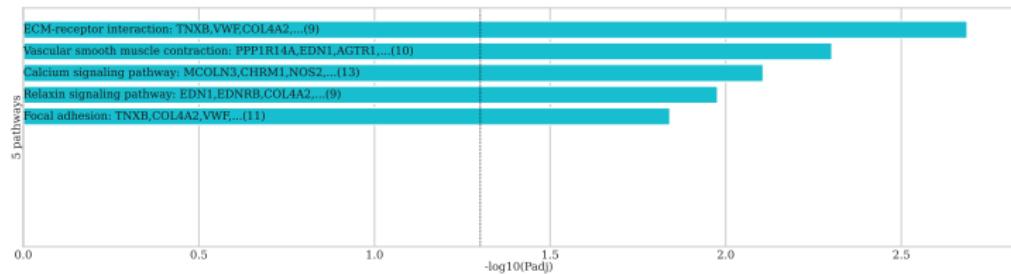


Figure: Down-regulated Pathways on Normal vs. Primary for Non-recur in LUAD

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

4.11. Discovery of Gene Fusion

Arriba?

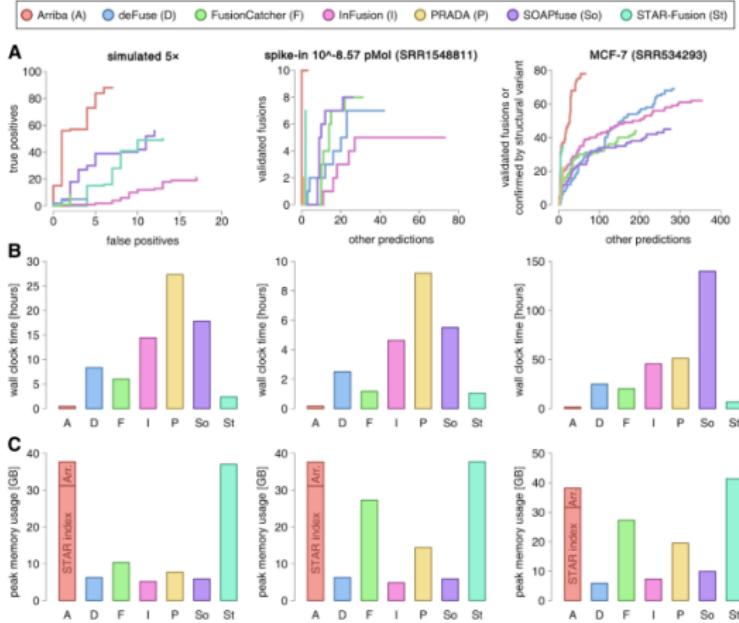


Figure: Benchmark of Arriba versus alternative methods (Uhrig et al., 2021)

Findings in Gene Fusion Discovery

5. Discussion

6. References

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