

# Lung Precancer Analysis

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# Overview

1 Introduction

2 Materials

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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

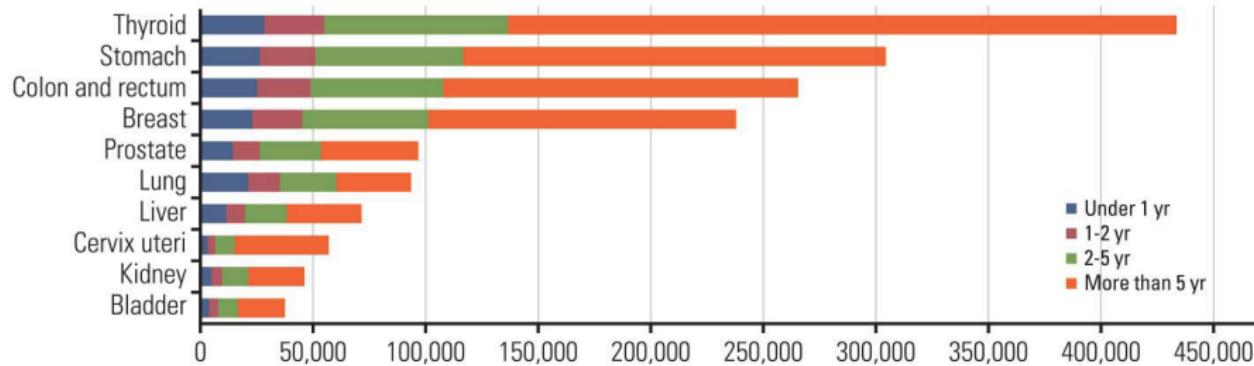


Figure: Common cancer survival rates (S. Hong et al., 2021)

## 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

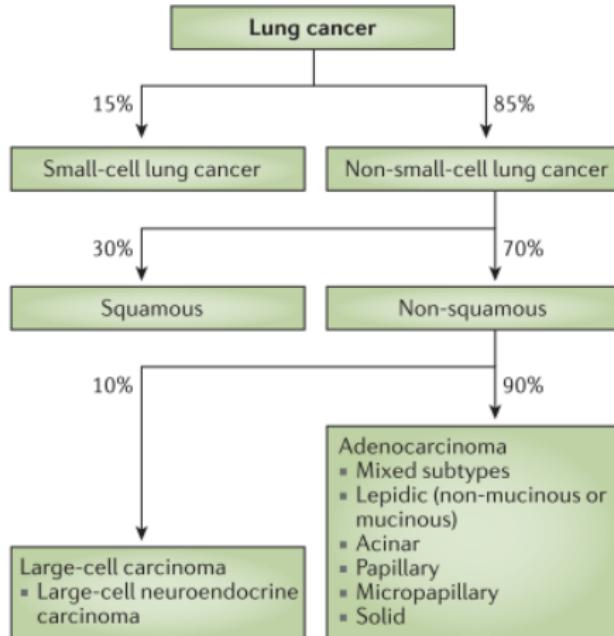


Figure: Lung cancer classification (Gridelli et al., 2015)

# 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

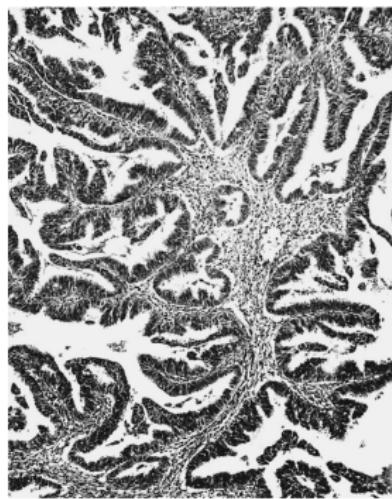
## TCGA LUSC (Dosenbach et al., 2007)

- 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

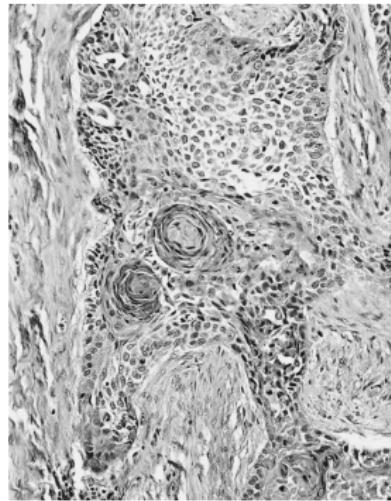
# 1. Introduction

## 1.5. LUAD vs. LUSC

# LUAD vs. LUSC I



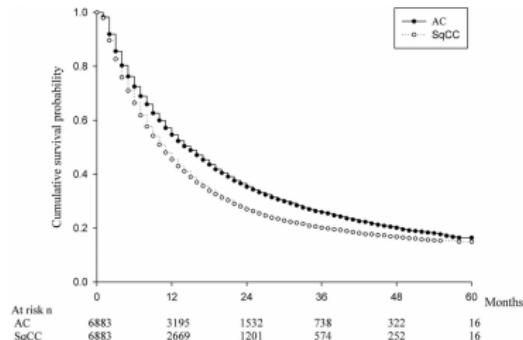
(a) LUAD



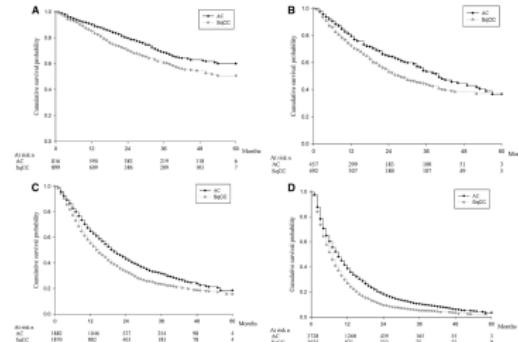
(b) LUSC

**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

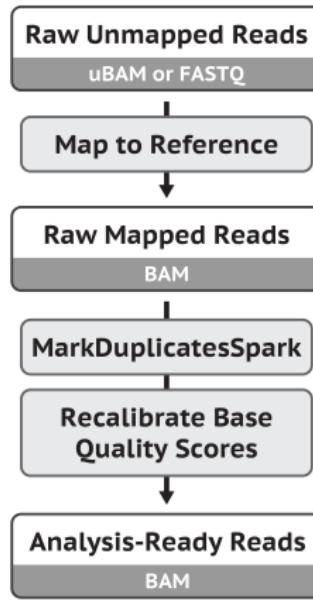
Smoking?	Stage	Number of Samples	
Never	Normal	10	
	AAH	1	
	CIS+AIS	3	
	Primary	4	
	Total	18	
Ex	Normal	3	
	CIS+AIS	1	
	Primary	1	
	Total	5	
Current	CIS+AIS	1	
	Primary	1	
	Total	2	

### 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

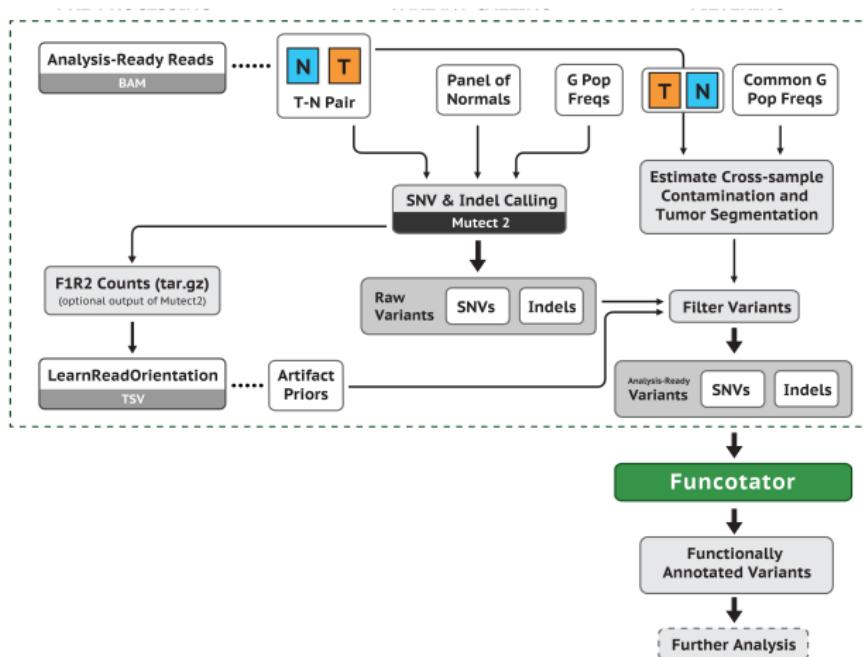


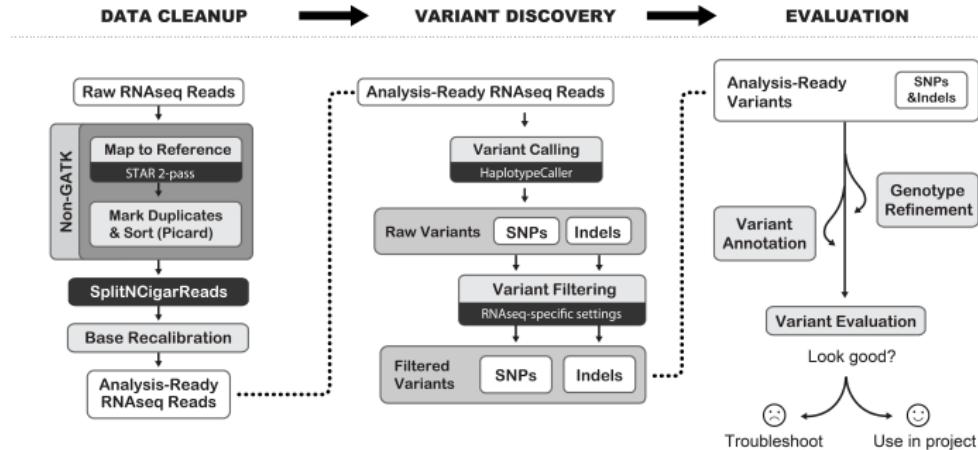
Figure: Somatic short variant (SNVs + Indels) discovery workflow (Van der Auwera et al., 2013; DePristo et al., 2011)

# 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?

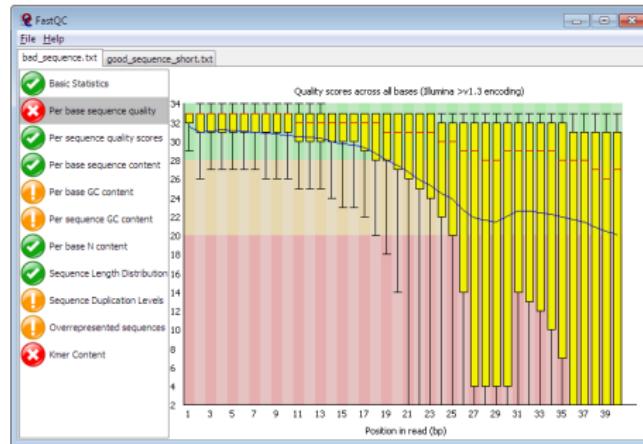


Figure: Example of FastQC Result (Andrews et al., 2012)

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

# FastQC on WES

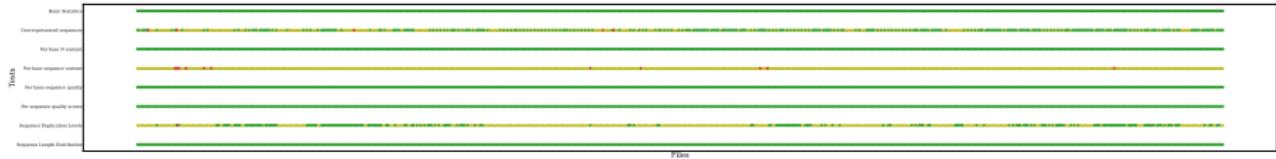
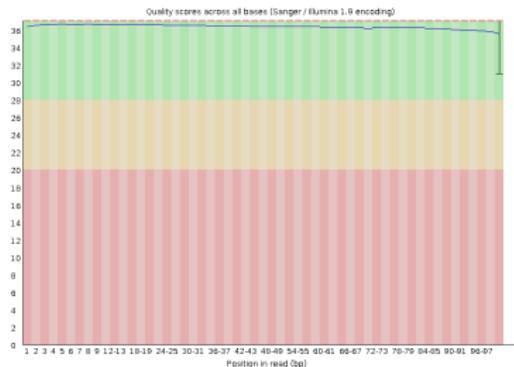


Figure: FastQC with WES data

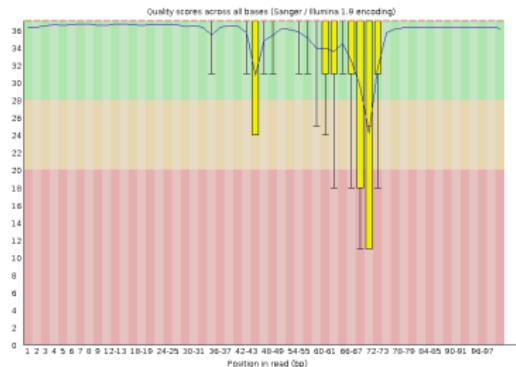
## Failure on 33P1 sample

33P1 is excluded from further analyses.

# Failure on 33P1 I



(a) 33N



(b) 33P1

Figure: Per Base Sequence Quality Results

# Failure on 33P1 II

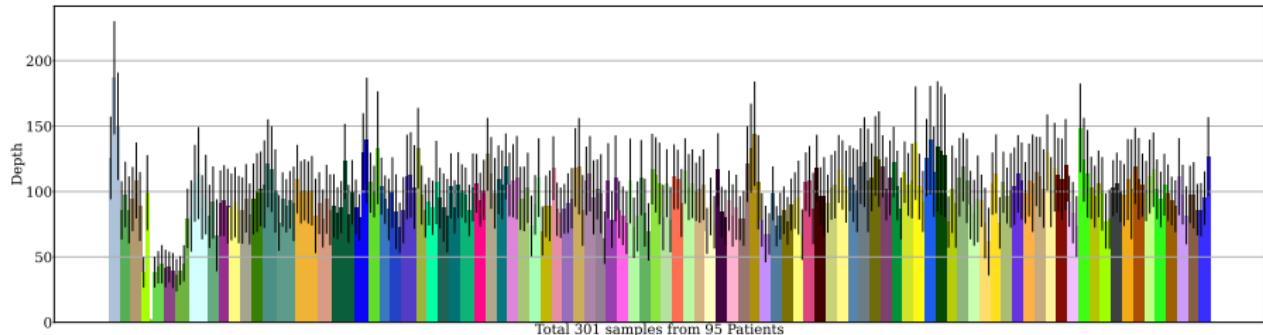


Figure: Coverage Depth Plot

- 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

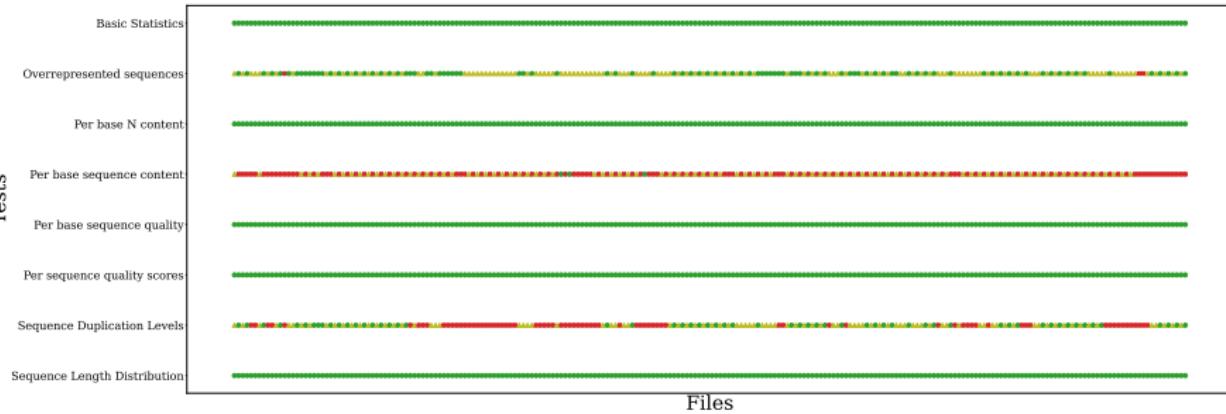


Figure: FastQC with WTS data

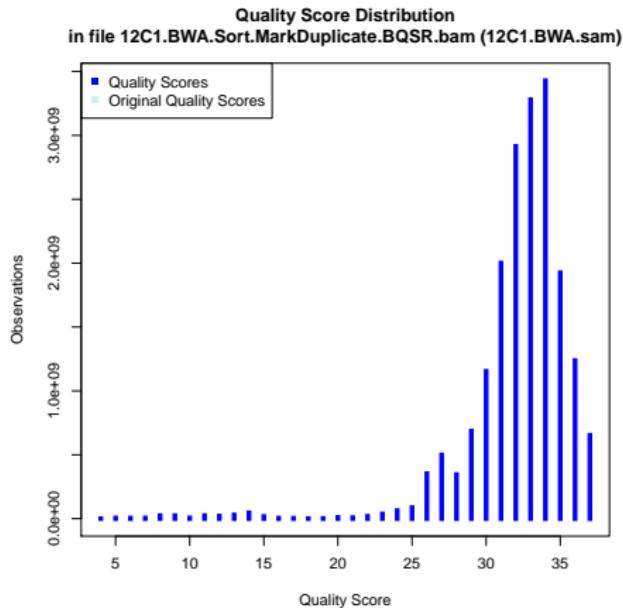
All sample are good to analysis

∴ 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

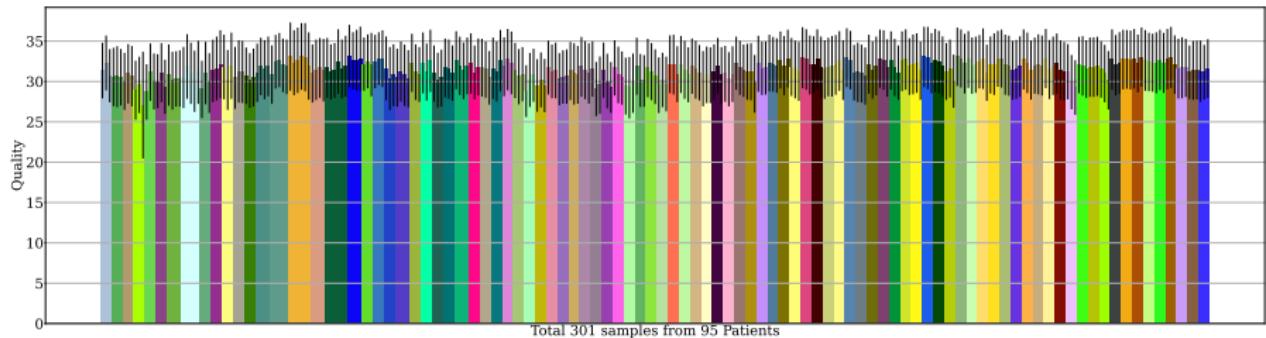


Figure: Quality Distribution by Samples

# Findings in Picard

## 4. Results

### 4.3. Copy Number Variation Analysis with PureCN

# PureCN?

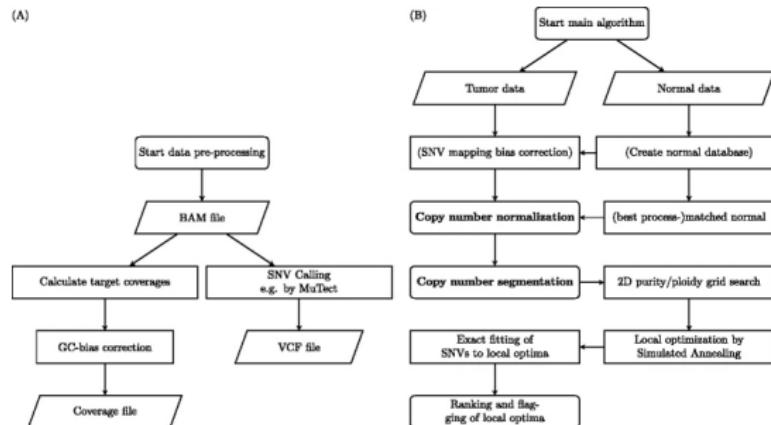
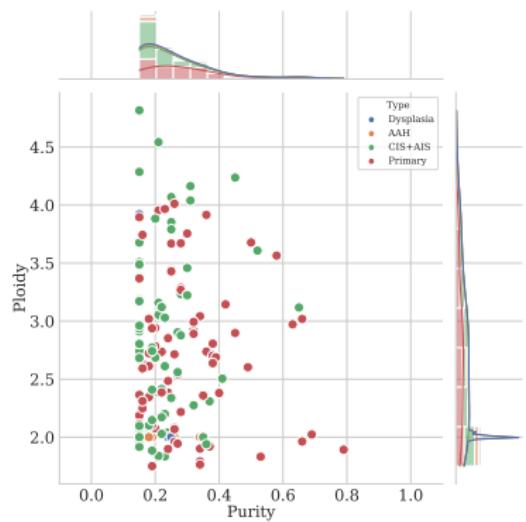
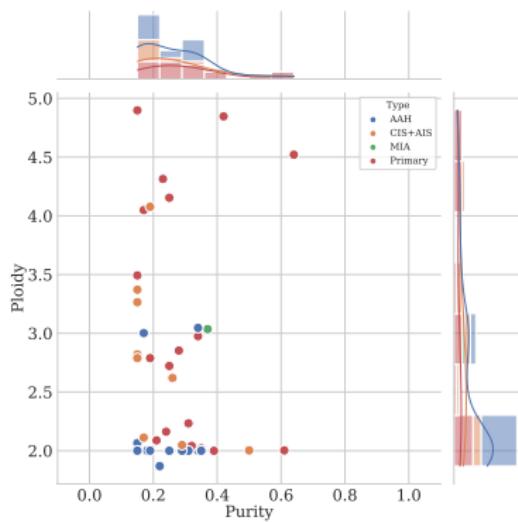


Figure: Flowchart of the PureCN data pre-processing pipeline (Riester et al., 2016)

# Purity & Ploidy on WES



(a) LUSC Samples



(b) LUAD Samples

Figure: Cellularity and Ploidy from PureCN

# LUSC in CNV Plot I

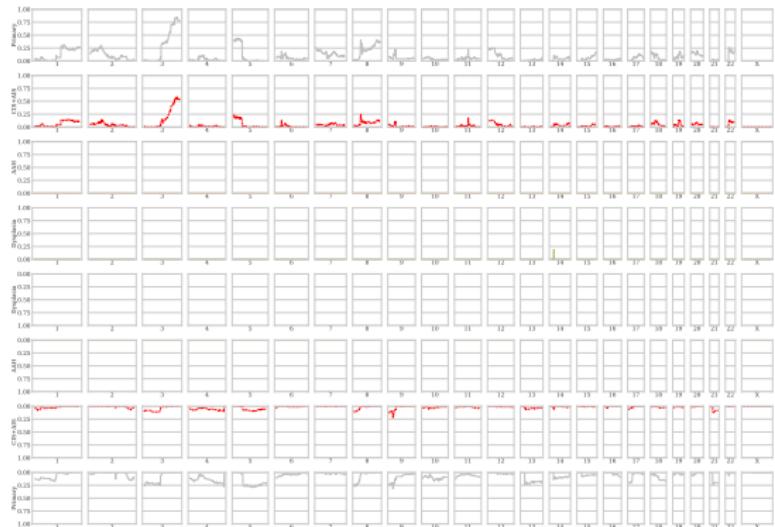


Figure: LUSC in CNV Plot

# 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

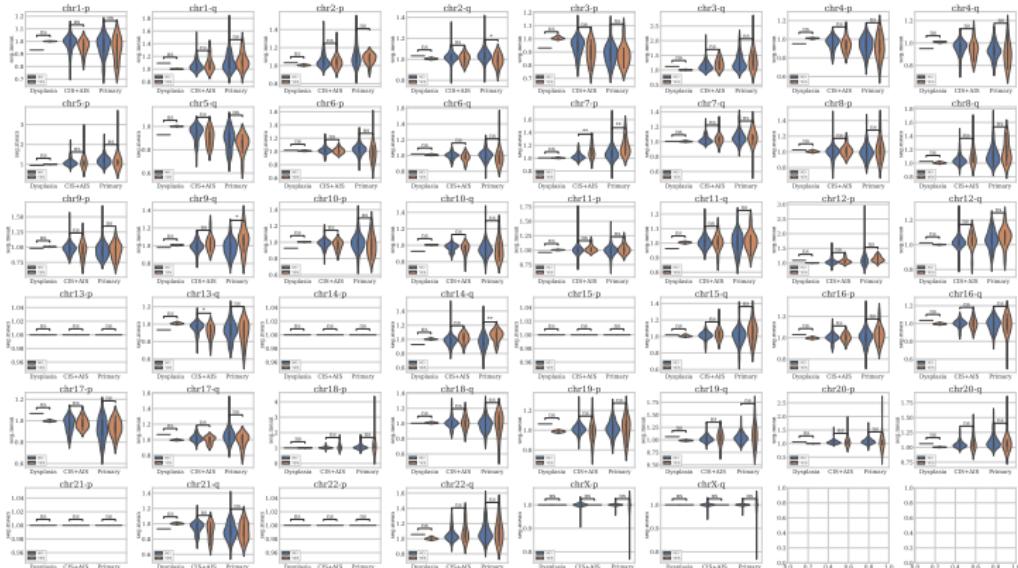


Figure: LUSC with Recurrence in Violin Plots

# LUSC with Smoking in CNV Plot

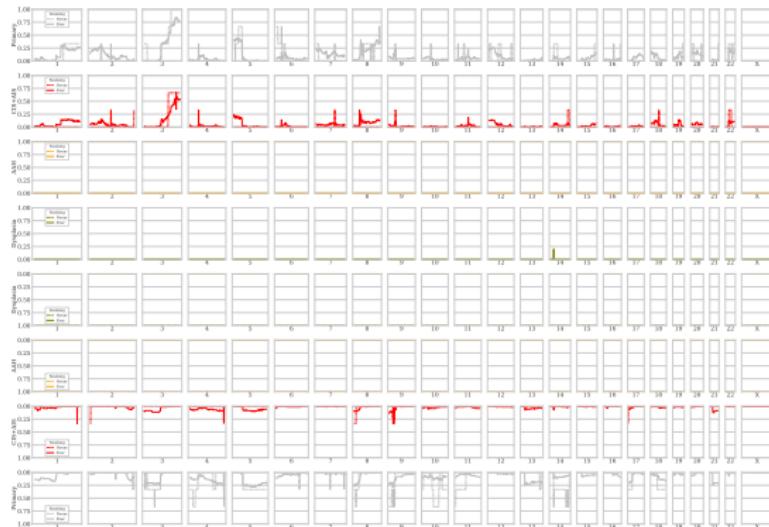


Figure: LUSC with Smoking in CNV Plot

# LUSC with Smoking in CNV Plot II

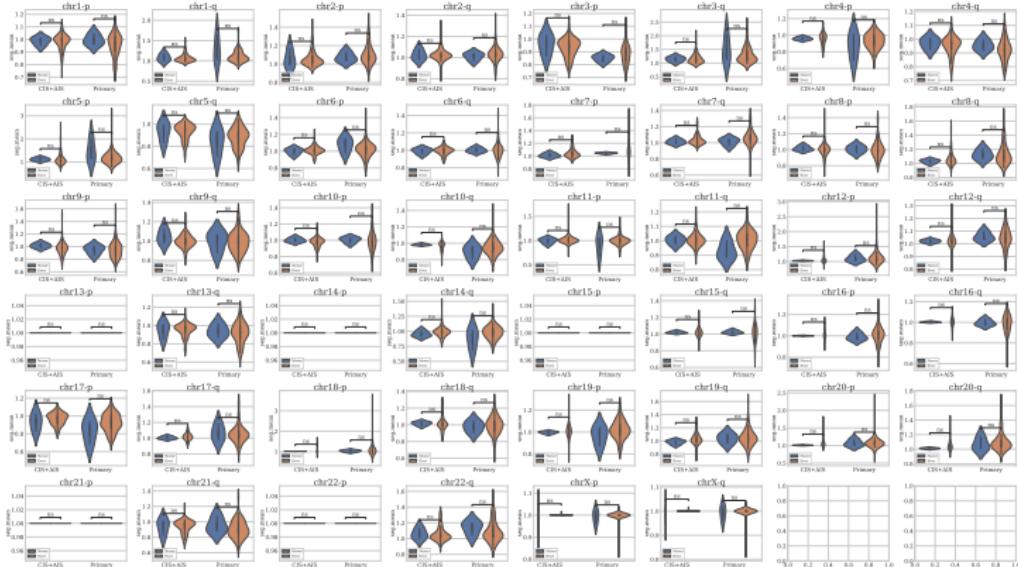


Figure: LUSC with Smoking in Violin Plots

# 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

- ① 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

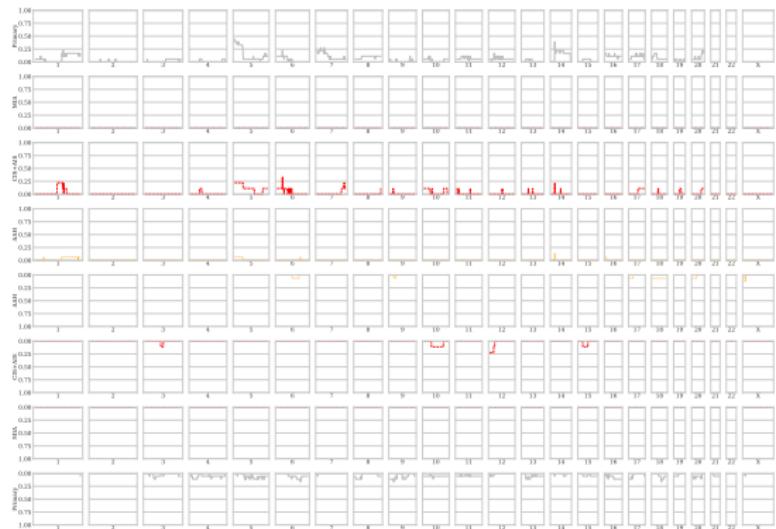


Figure: LUAD in CNV Plot

# LUAD in CNV Plot II

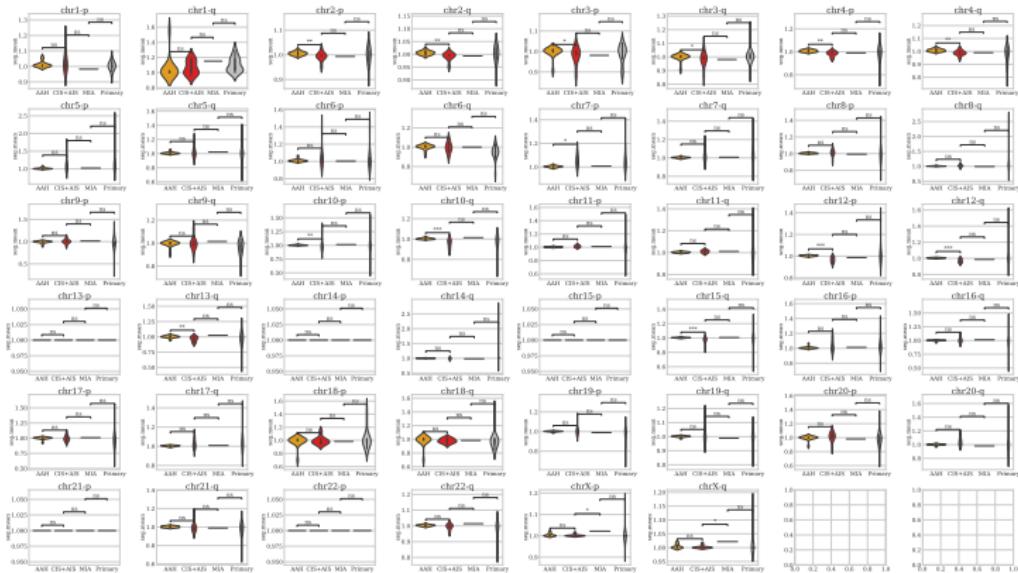


Figure: LUAD in Violin Plots

# LUAD with Recurrence in CNV Plot

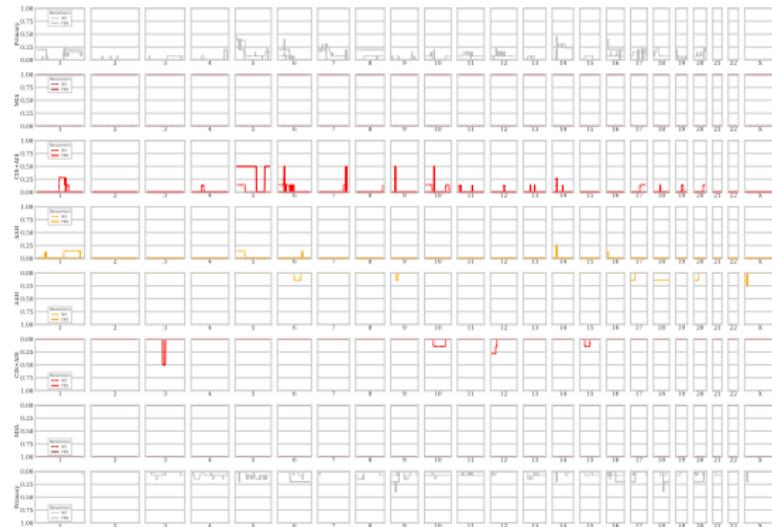


Figure: LUAD with Recurrence in CNV Plot

# 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

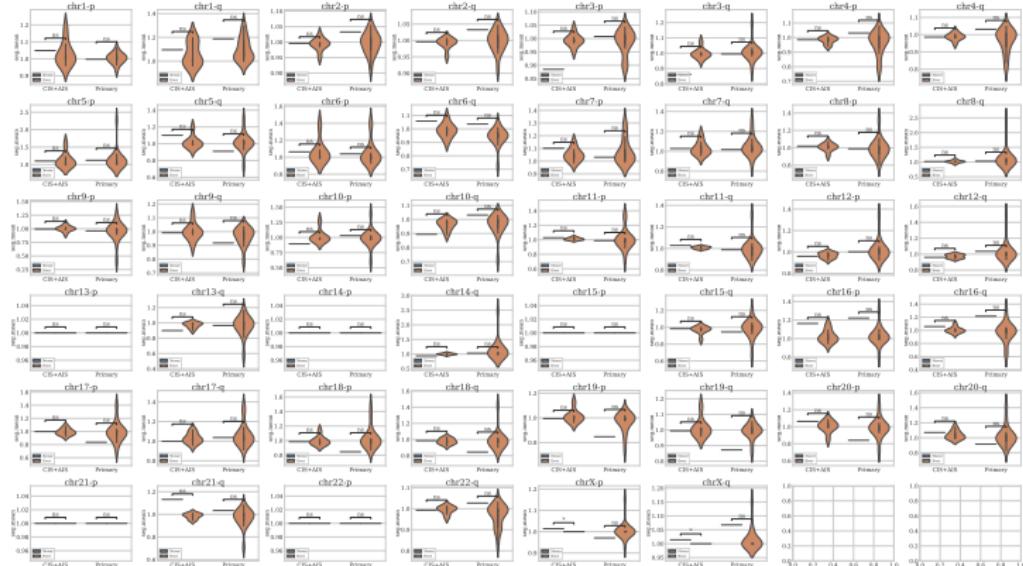


Figure: LUAD with Smoking in Violin Plots

# 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

- ① 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

## 4. Results

### 4.4. Copy Number Variation Analysis with Gistic

# Gistic?

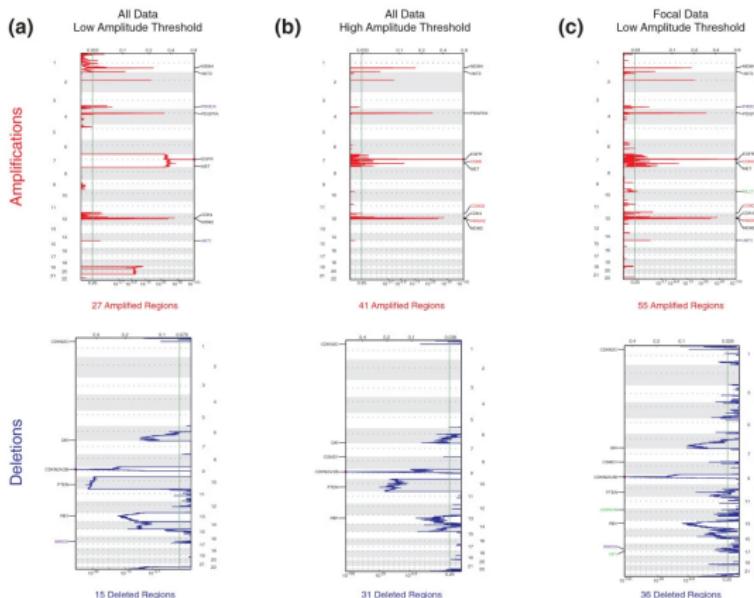


Figure: Effects of arm-level events on GISTIC results (Mermel et al., 2011)

## 4. Results

### 4.4. Copy Number Variation Analysis with Gistic

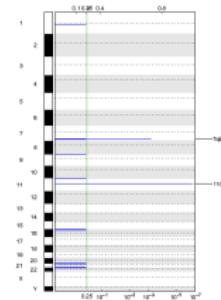
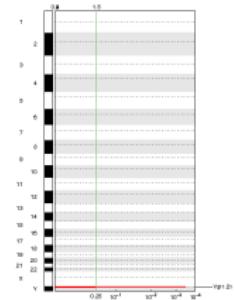
#### 4.4.1. Gistic in LUSC

# LUSC Data Composition

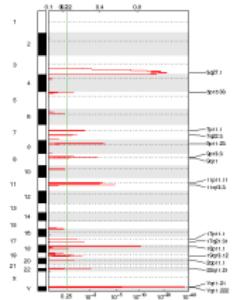
Table: Number of WES samples

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

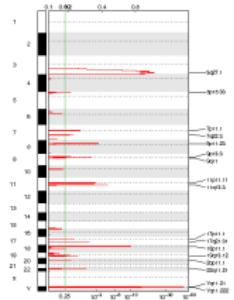
# Gistic in LUSC



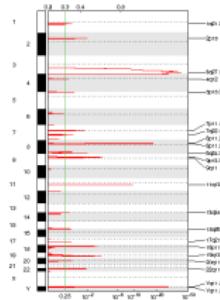
(a) Dysplasia



(b) CIS



(c) Precancer

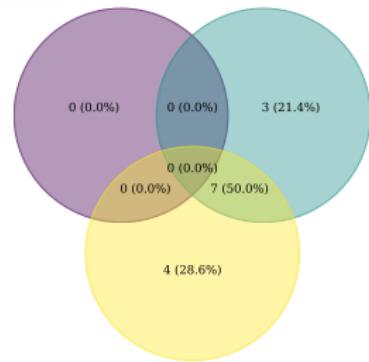


(d) Primary

Figure: Gistic results in LUSC

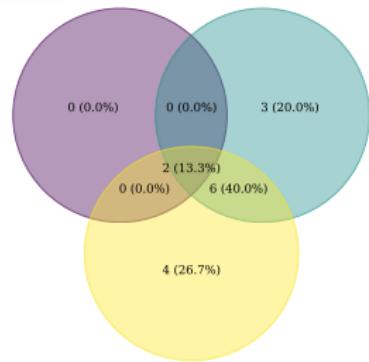
# Peaks in LUSC I

Dysplasia  
CIS  
Primary



(a) Amplification

Dysplasia  
CIS  
Primary



(b) Deletion

Figure: Venn Diagram among Peaks in LUSC – stage

# Peaks in LUSC II

Table: Amplification Peaks in LUSC – stage

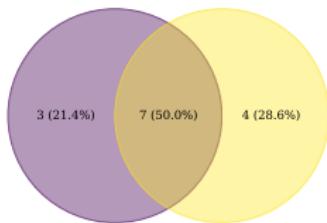
Peaks	Dysplasia	CIS	Primary	CGC Genes
1q21.3			*	ARNT,MLLT11,S100A7,SETDB1,TPM3
2p15			*	XPO1
3q27.1	*	*		
4q12			*	CHIC2,FIP1L1,KDR,KIT,PDGFRA
5p15.33	*	*		SDHA,TERT
7p11.1	*	*		
7q22.1			*	CUX1,TRRAP
7q22.3	*			
8p11.21			*	ANK1,HOOK3,IKBKB,KAT6A
8p11.23	*	*		FGFR1,NSD3
8q24.3			*	RECQL4
9p13.3	*	*		FANCG
9q11	*	*		
11p11.11	*			
11q13.3	*	*		CCND1
13q34			*	
15q26.3			*	
17p11.1	*			
17q21.31	*	*		BRCA1,ETV4
18p11.1	*	*		
19q13.2			*	AKT2,CD79A,CIC
19q13.12	*			
20p11.1	*			
20q11.22			*	
22q11.21	*	*		CLTC1,DGCR8,LZTR1,SEPT5

# Peaks in LUSC III

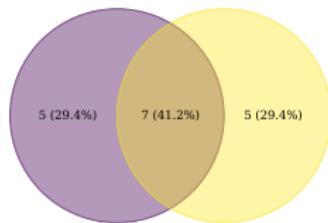
Table: Deletion Peaks in LUSC – stage

Peaks	Dysplasia	CIS	Primary	CGC Genes
1p36.11		*		ARID1A,MDS2
1p36.13		*		ARHGEF10L,PAX7,SDHB,SPEN
1q21.3	*	*		ARNT,MLLT11,S100A7,SETDB1,TPM3
1q25.3		*		
2p25.3		*		
2q36.3		*		
3p12.1		*		
4q13.3		*		
4q31.3		*		FBXW7
4q35.2		*		DUX4L,FAT1
5q21.1		*		
5q35.3	*	*		FLT4,NSD1
6p21.32		*	*	DAXX
7q34	*	*	*	BRAF,FAM131B,KIAA1549,TRIM24
7q35		*	*	CNTNAP2
8p23.1		*	*	
9p11.2		*	*	
9p21.3		*	*	CDKN2A,MLLT3
10q22.1		*	*	PRF1
11p15.4		*	*	CARS,LMO1,NUP98
11q11	*	*	*	
15q11.2		*		
16p11.2		*		FUS
17p11.2		*		FLCN,NCOR1,SPECC1
17q21.31		*		BRCA1,ETV4
18q11.1		*	*	
18q23		*		
19q13.31		*		

# Peaks in LUSC IV



(a) Amplification



(b) Deletion

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

# Peaks in LUSC V

Table: Amplification Peaks in LUSC – PRE vs. PRI

Peaks	Precancer	Primary	CGC Genes
1q21.3		*	ARNT,MLLT11,S100A7,SETDB1,TPM3
2p15		*	XPO1
3q27.1	*	*	
4q12		*	CHIC2,FIP1L1,KDR,KIT,PDGFRA
5p15.33	*	*	SDHA,TERT
7p11.1	*	*	
7q22.1		*	CUX1,TRRAP
7q22.3	*		
8p11.21		*	ANK1,HOOK3,IKBKB,KAT6A
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,CD79A,CIC
19q13.12	*		
20p11.1	*		
20q11.22		*	
22q11.21	*	*	CLTCL1,DGCR8,LZTR1,SEPT5

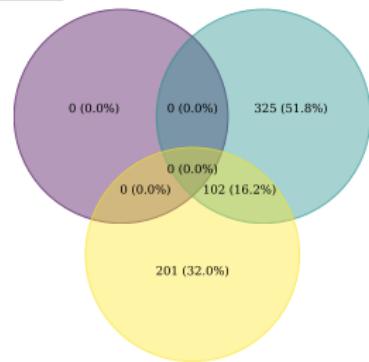
# Peaks in LUSC VI

Table: Deletion Peaks in LUSC – PRE vs. PRI

Peaks	Precancer	Primary	CGC Genes
1p36.11	*		ARID1A,MDS2
1p36.13	*		ARHGEF10L,PAX7,SDHB,SPEN
1q21.3	*	*	ARRNT,MLLT11,S100A7,SETDB1,TPM3
1q25.3		*	
2p25.3		*	
2q36.3		*	
3p12.1		*	
4q13.3		*	
4q31.3		*	FBXW7
4q35.2		*	DUX4L1,FAT1
5q21.1		*	
5q35.3	*	*	FLT4,NSD1
6p21.32	*	*	DAXX
6p25.3	*		IRF4
7q34	*	*	BRAF,FAM131B,KIAA1549,TRIM24
7q35	*	*	CNTNAP2
8p23.1	*	*	
9p11.2	*	*	
9q21.3	*	*	CDKN2A,MLLT3
10q22.1	*	*	PRF1
11p15.4	*	*	CARS,LMO1,NUP98
11q11	*	*	
15q11.2	*	*	
16p11.2	*		FUS
17p11.2	*		FLCN,NCOR1,SPECC1
17q12	*		CDK12,ERBB2,LASP1,MLLT6,TAF15
17q21.31	*		BRCA1,ETV4
18q11.1	*	*	
18q23		*	
19q13.31	*		

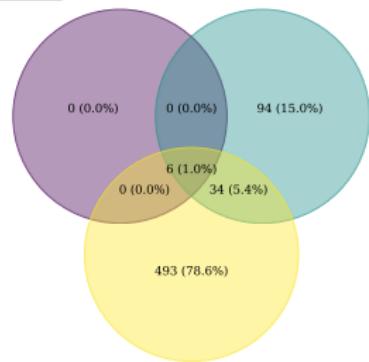
# Genes in LUSC I

Dysplasia  
CIS  
Primary



(a) Amplification

Dysplasia  
CIS  
Primary



(b) Deletion

Figure: Venn Diagram among Genes in LUSC – stage

# Genes in LUSC II

Table: Amplification Genes in LUSC – stage

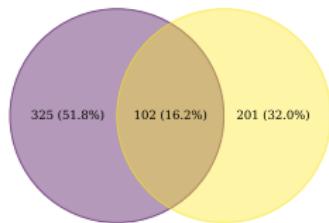
CGC Genes	Dysplasia	CIS	Primary
AKT2	*		
ANK1		*	
BCL11A		*	
CCND1	*		
CEBPA	*		
CEP89	*		
DGCR8	*	*	
FANCG	*		
FGFR1	*	*	
HOOK3		*	
IKBKB		*	
KAT6A		*	
LSM14A	*		
LZTR1	*	*	
PAX5	*		
PIK3CA	*		
RECQL4		*	
REL		*	
SOX2	*		
XPO1		*	

# Genes in LUSC III

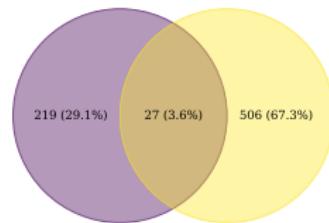
Table: Deletion Genes in LUSC – stage

CGC Genes	Dysplasia	CIS	Primary
ACKR3			*
ACSL3			*
ARHGEF10			*
ATIC			*
BARD1			*
CASP3			*
CDKN2A	*		*
CREB1			*
CUL3			*
ERBB4			*
FAT1			*
FEV			*
IDH1			*
MLLT3	*		
PAX3			*

# Genes in LUSC IV



(a) Amplification



(b) Deletion

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

# Genes in LUSC V

Table: Amplification Genes in LUSC – PRE vs. PRI

CGC Genes	Precancer	Primary
AKT2	*	
ANK1		*
BCL11A		*
CCND1	*	
CEBPA	*	
CEP89	*	
DGCR8	*	*
FANCG	*	
FGFR1	*	*
HOOK3		*
IKBKB		*
KAT6A		*
LSM14A	*	
LZTR1	*	*
PAX5	*	
PIK3CA	*	
RECQL4		*
REL		*
SOX2	*	
XPO1		*

# Genes in LUSC VI

Table: Deletion Genes in LUSC – PRE vs. PRI

CGC Genes	Precancer	Primary
ACKR3		*
ACSL3		*
ARHGEF10		*
ATIC		*
BARD1		*
CASP3		*
CD274	*	
CDKN2A	*	*
CREB1		*
CUL3		*
ERBB4		*
FAT1		*
FEV		*
IDH1		*
JAK2	*	
MLLT3	*	
NFIB	*	
PAX3		*
PDCD1LG2	*	
PSIP1	*	
PTPRD	*	

# Findings in LUSC Gistic Results

## 4. Results

### 4.4. Copy Number Variation Analysis with Gistic

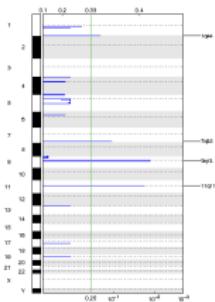
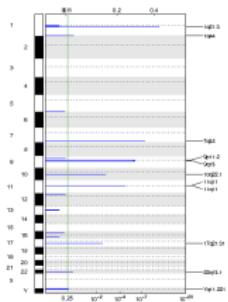
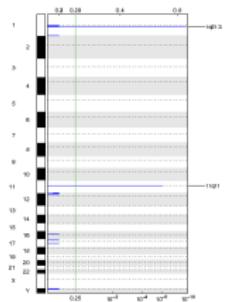
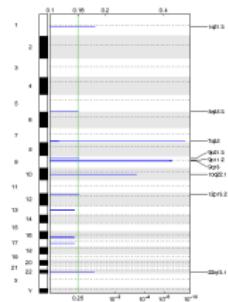
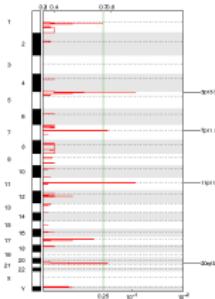
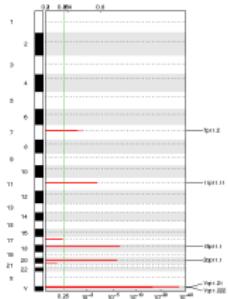
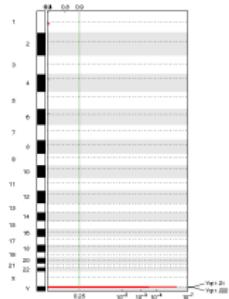
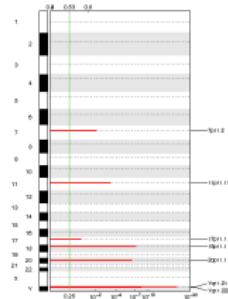
#### 4.4.2. Gistic in LUAD

# 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

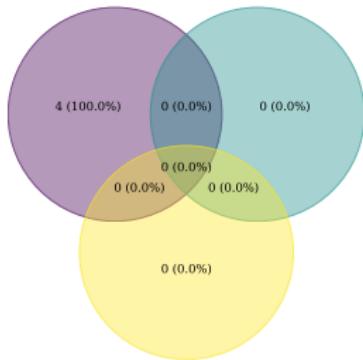
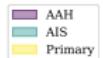
(b) AIS

(c) Precancer

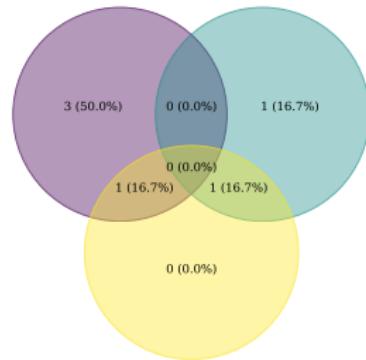
(d) Primary

Figure: Gistic results in LUAD

# Peaks in LUAD I



(a) Amplification



(b) Deletion

Figure: Venn Diagram among Peaks in LUAD – stage

# Peaks in LUAD II

Table: Amplification Peaks in LUAD – stage

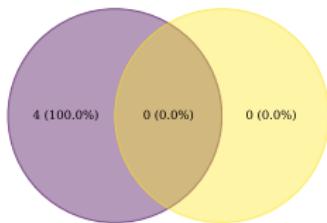
Peaks	AAH	AIS	Primary	CGC Genes
5p15.33			*	SDHA,TERT
7p11.1			*	
7p11.2	*			EGFR,ZNF479
11p11.11	*		*	
17p11.1	*			
18p11.1	*			
20p11.1	*			
20q13.33			*	PTK6,SS18L1

# Peaks in LUAD III

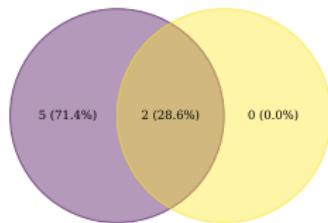
Table: Deletion Peaks in LUAD – stage

Peaks	AAH	AIS	Primary	CGC Genes
1q21.3	*	*		ARNT,MLLT11,S100A7,SETDB1,TPM3
1q44			*	AKT3
5q35.3	*			FLT4,NSD1
7q35	*		*	CNTNAP2
9p11.2	*			
9p21.3	*			CDKN2A,MLLT3
9q13	*		*	
10q22.1	*			PRF1
11q11		*	*	
12p13.2	*			ETV6
22q13.1	*			APOBEC3B,MRTFA,PDGFB

# Peaks in LUAD IV



(a) Amplification



(b) Deletion

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

# Peaks in LUAD V

Table: Amplification Peaks in LUAD – PRE vs. PRI

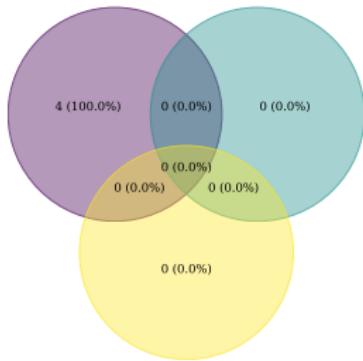
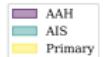
Precancer Peaks	Primary	CGC Genes
5p15.33	*	SDHA,TERT
7p11.1	*	
7p11.2	*	EGFR,ZNF479
11p11.11	*	*
18p11.1	*	
20p11.1	*	
20q13.33	*	PTK6,SS18L1

# Peaks in LUAD VI

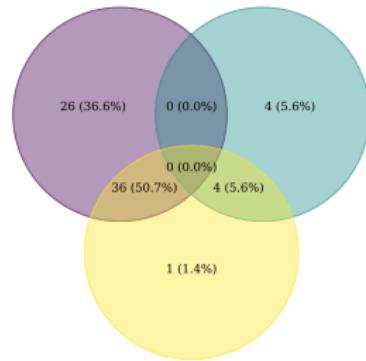
Table: Deletion Peaks in LUAD – PRE vs. PRI

Peaks	Precancer	Primary	CGC Genes
1q21.3	*		ARNT,MLLT11,S100A7,SETDB1,TPM3
1q44	*	*	AKT3
7q35	*	*	CNTNAP2
9p11.2	*		
9q13	*	*	
10q22.1	*		PRF1
11q11	*	*	
17q21.31	*		BRCA1,ETV4
22q13.1	*		APOBEC3B,MRTFA,PDGFB

# Genes in LUAD I



(a) Amplification



(b) Deletion

Figure: Venn Diagram among Genes in LUAD – stage

# Genes in LUAD II

Table: Amplification Genes in LUAD – stage

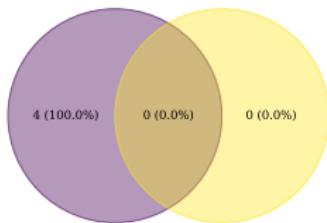
Genes	AAH	AIS	Primary
FRG1CP	*		
LOC644669	*		
LOC646813	*		
ZNF716	*		

## Genes in LUAD III

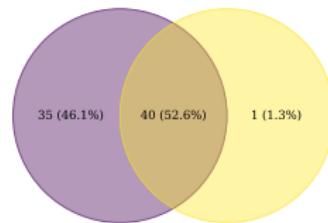
Table: Deletion Genes in LUAD – stage

Genes	Adm	AS	Primary
ANKR030A1	*	*	
ANKR030A2	*	*	
ANKR030A3	*	*	
ANKR030A4	*	*	
AQTP7P3			
ARHGEF14P			
ATM			
C9orf65			
C9orf66	*	*	
C9orf67	*	*	
C9orf68	*	*	
COL11A1			
CT42A4			
CT42A5			
DMWD2C			
DMD			
FAM104A1			
FAM104A2			
FAM104A3			
FAM104A4			
FAM104A5			
FAM104B1			
FAM104B2			
FAM104B3			
FAM104B4			
FAM104B5			
FAM104B6			
FAM104B7			
FAM104B8			
FAM104B9			
FAM104B10			
FAM104B11			
FAM104B12			
FAM104B13			
FAM104B14			
FAM104B15			
FAM104B16			
FAM104B17			
FAM104B18			
FAM104B19			
FAM104B20			
FAM104B21			
FAM104B22			
FAM104B23			
FAM104B24			
FAM104B25			
FAM104B26			
FAM104B27			
FAM104B28			
FAM104B29			
FAM104B30			
FAM104B31			
FAM104B32			
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FAM104B396			
FAM104B397			
FAM104B398			
FAM104B399			
FAM104B400			
FAM104B401			
FAM104B402			
FAM1			

# Genes in LUAD IV



(a) Amplification



(b) Deletion

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

# Genes in LUAD V

Table: Amplification Genes in LUAD – PRE vs. PRI

Genes	Precancer	Primary
FRG1CP	*	
LOC644669	*	
LOC646813	*	
ZNF733P	*	

## Genes in LUAD VI

Table: Deletion Genes in LUAD – PRE vs. PRI

# Findings in LUAD Gistic Results

## 4. Results

### 4.4. Copy Number Variation Analysis with Gistic

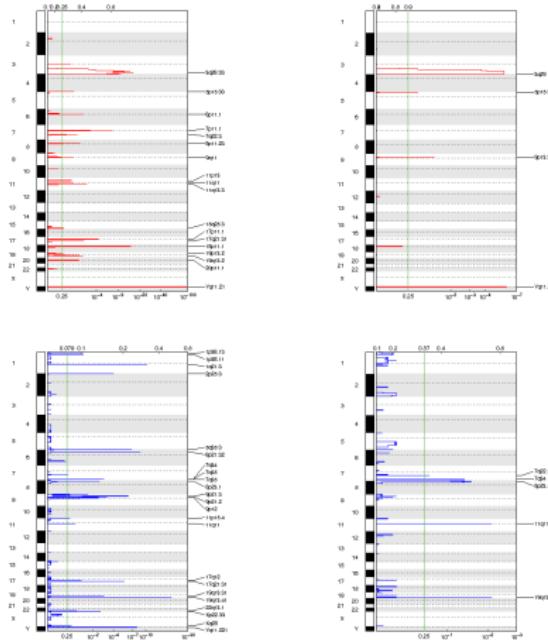
#### 4.4.3. Gistic in Recurrence & LUSC

# 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	

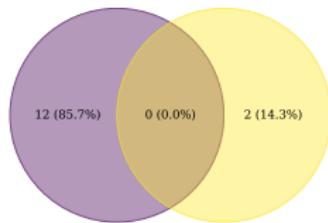
# Gistic in Recurrence & LUSC – CIS



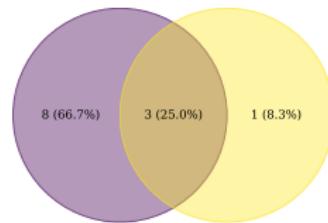
(a) Non-recurrence    (b) Recurrence

Figure: Gistic results in Recurrence & LUSC – CIS

# Peaks in Recurrence & LUSC – CIS I



(a) Amplification



(b) Deletion

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

# Peaks in Recurrence & LUSC – CIS II

Table: Amplification Peaks in Recurrence & LUSC – CIS

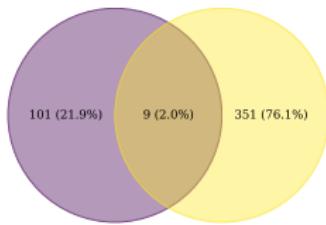
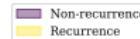
Peaks	Non-recurrence	Recurrence	CGC Genes
3q26.33	*		SOX2
3q29		*	MB21D2,MUC4,TFRC
5p15.33	*	*	SDHA,TERT
6p11.1	*		
7p11.1	*		
7q22.3	*		
8p11.23	*		FGFR1,NSD3
9p13.3		*	FANCG
9q11	*		
11p13	*		LMO2,WT1
11q11	*		
11q13.3	*		CCND1
15q25.3	*		NTRK3
17p11.1	*		
17q21.31	*		BRCA1,ETV4
18p11.1	*		
19p13.2	*		CD209,DNM2,KEAP1,MUC16,SMARCA4
19q13.2	*		AKT2,CD79A,CIC
20p11.1	*		

# Peaks in Recurrence & LUSC – CIS III

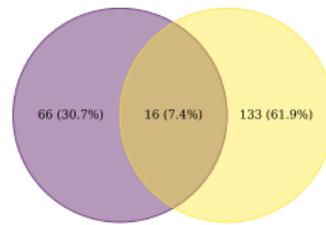
Table: Deletion Peaks in Recurrence & LUSC – CIS

Peaks	Non-recurrence	Recurrence	CGC Genes
1p36.11	*		ARID1A,MDS2
1p36.13	*		ARHGEF10L,PAX7,SDHB,SPEN
1q21.3	*		ARNT,MLLT11,S100A7,SETDB1,TPM3
2p25.3	*		
5q35.3	*		FLT4,NSD1
6p21.32	*		DAXX
7q22.1		*	CUX1,TRRAP
7q34	*	*	BRAF,FAM131B,KIAA1549,TRIM24
7q35	*		CNTNAP2
8p23.1	*	*	
9p12	*		
9p21.2	*		
9p21.3	*		CDKN2A,MLLT3
11p15.4	*		CARS,LMO1,NUP98
11q11	*	*	
17q12	*		CDK12,ERBB2,LASP1,MLLT6,TAF15
17q21.31	*		BRCA1,ETV4
19q13.31	*		
19q13.41	*	*	PPP2R1A
22q13.1	*		APOBEC3B,MRTFA,PDGFB

# Genes in Recurrence & LUSC – CIS I



(a) Amplification



(b) Deletion

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

# Genes in Recurrence & LUSC – CIS II

Table: Amplification Genes in Recurrence & LUSC – CIS

CGC Genes	Non-recurrence	Recurrence
BCL6		*
CCND1	*	
EIF4A2		*
ETV5		*
FANCG		*
FGFR1	*	
IGF2BP2		*
LPP		*
MAP3K13		*
MB21D2		*
MECOM		*
MUC4		*
PIK3CA		*
SOX2	*	*
TBL1XR1		*
TFRC		*
TP63		*

# Genes in Recurrence & LUSC – CIS III

Table: Deletion Genes in Recurrence & LUSC – CIS

CGC Genes	Non-recurrence	Recurrence
ARHGEF10		*
CDKN2A	*	

# Gistic in Recurrence & LUSC – Precancer

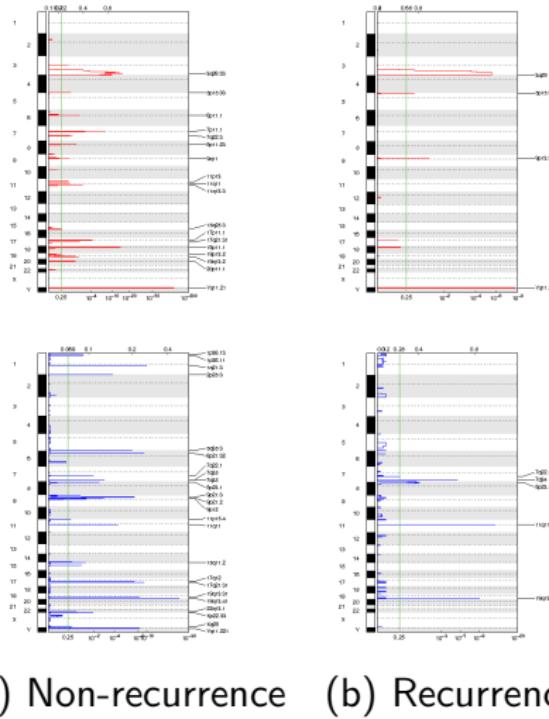
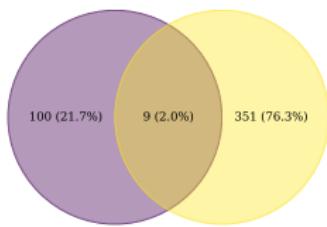
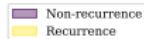
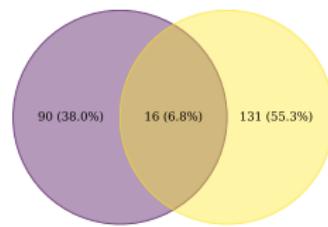


Figure: Gistic results in Recurrence & LUSC – Precancer

# Peaks in Recurrence & LUSC – Precancer I



(a) Amplification



(b) Deletion

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

# Peaks in Recurrence & LUSC – Precancer II

Table: Amplification Peaks in Recurrence & LUSC – Precancer

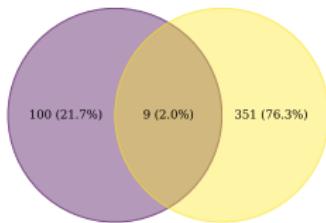
Peaks	Non-recurrence	Recurrence	CGC Genes
3q26.33	*		SOX2
3q29		*	MB21D2,MUC4,TFRC
5p15.33	*	*	SDHA,TERT
6p11.1	*		
7p11.1	*		
7q22.3	*		
8p11.23	*		FGFR1,NSD3
9p13.3		*	FANCG
9q11	*		
11p13	*		LMO2,WT1
11q11	*		
11q13.3	*		CCND1
15q25.3	*		NTRK3
17p11.1	*		
17q21.31	*		BRCA1,ETV4
18p11.1	*		
19p13.2	*		CD209,DNM2,KEAP1,MUC16,SMARCA4
19q13.2	*		AKT2,CD79A,CIC
20p11.1	*		

# Peaks in Recurrence & LUSC – Precancer III

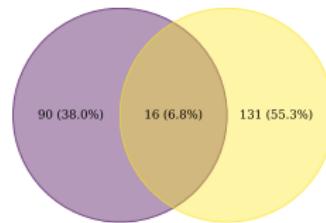
Table: Deletion Peaks in Recurrence & LUSC – Precancer

Peaks	Non-recurrence	Recurrence	CGC Genes
1p36.11	*		ARID1A,MDS2
1p36.13	*		ARRHGEF10L,PAX7,SDHB,SPEN
1q21.3	*		ARNT,MLLT11,S100A7,SETDB1,TPM3
2p25.3	*		
5q35.3	*		FLT4,NSD1
6p21.32	*		DAXX
7q22.1	*	*	CUX1,TRRAP
7q34		*	BRAF,FAM131B,KIAA1549,TRIM24
7q35	*		CNTNAP2
8p23.1	*	*	
9p12	*		
9p21.2	*		
9p21.3	*		CDKN2A,MLLT3
11p15.4	*		CARS,LMO1,NUP98
11q11	*	*	
15q11.2	*		
17q12	*		CDK12,ERBB2,LASP1,MLLT6,TAF15
17q21.31	*		BRCA1,ETV4
19q13.31	*		
19q13.41	*	*	PPP2R1A
22q13.1	*		APOBEC3B,MRTFA,PDGFB

# Genes in Recurrence & LUSC – Precancer I



(a) Amplification



(b) Deletion

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

# Genes in Recurrence & LUSC – Precancer II

Table: Amplification Genes in Recurrence & LUSC – Precancer

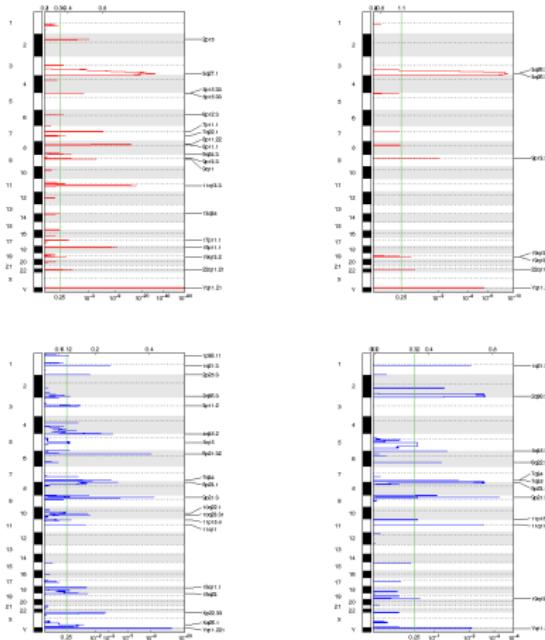
CGC Genes	Non-recurrence	Recurrence
BCL6		*
CCND1	*	
EIF4A2		*
ETV5		*
FANCG		*
FGFR1	*	
IGF2BP2		*
LPP		*
MAP3K13		*
MB21D2		*
MECOM		*
MUC4		*
PIK3CA		*
SOX2	*	*
TBL1XR1		*
TFRC		*
TP63		*

# Genes in Recurrence & LUSC – Precancer III

Table: Deletion Genes in Recurrence & LUSC – Precancer

CGC Genes	Non-recurrence	Recurrence
ARHGEF10		*
CDKN2A	*	

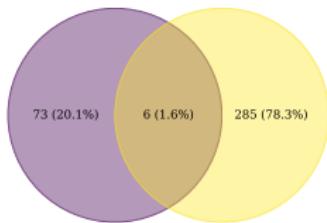
Gistic in Recurrence & LUSC – Primary



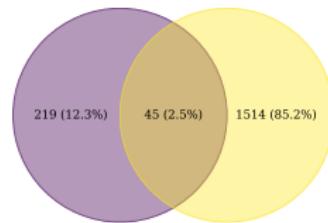
(a) Non-recurrence (b) Recurrence

## Figure: Gistic results in Recurrence & LUSC – Primary

# Peaks in Recurrence & LUSC – Primary I



(a) Amplification



(b) Deletion

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

# Peaks in Recurrence & LUSC – Primary II

Table: Amplification Peaks in Recurrence & LUSC – Primary

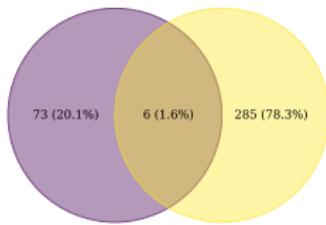
Peaks	Non-recurrence	Recurrence	CGC Genes
2p15	*		XPO1
3q26.33		*	SOX2
3q27.1	*		
5p15.33	*		SDHA,TERT
6p12.3	*		
7p11.1	*		
7q22.1	*		CUX1,TRRAP
8p11.1	*		
8p11.22	*		
8q24.3	*		RECQL4
9p13.3	*	*	FANCG
9q11	*		
11q13.3	*		CCND1
13q34	*		
17p11.1	*		
18p11.1	*		
19q13.2	*	*	AKT2,CD79A,CIC
22q11.21	*	*	CLTC1,DGCR8,LZTR1,SEPT5

# Peaks in Recurrence & LUSC – Primary III

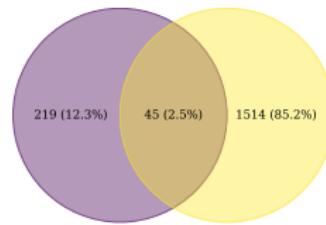
Table: Deletion Peaks in Recurrence & LUSC – Primary

Peaks	Non-recurrence	Recurrence	CGC Genes
1p36.11	*		ARID1A, MDS2
1q21.3	*	*	ARNT, MLLT11, S100A7, SETDB1, TPM3
2p25.3	*		
2q36.3	*	*	
3p11.2	*		
4q35.2	*		DUX4L1, FAT1
5q15	*		
5q35.3		*	FLT4, NSD1
6p21.32	*		DAXX
6q22.31		*	
7q34	*	*	BRAF, FAM131B, KIAA1549, TRIM24
7q35		*	CNTNAP2
8p23.1	*	*	
9p21.3	*	*	CDKN2A, MLLT3
10q22.1	*		PRF1
10q23.31	*		FAS, PTEN
11p15.4	*		CARS, LMO1, NUP98
11p15.5		*	HRAS
11q11	*	*	
18q11.1	*		
18q23	*		
19q13.43		*	

# Genes in Recurrence & LUSC – Primary I



(a) Amplification



(b) Deletion

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

# Genes in Recurrence & LUSC – Primary II

Table: Amplification Genes in Recurrence & LUSC – Primary

CGC Genes	Non-recurrence	Recurrence
BCL11A	*	
BCL6		*
EIF4A2		*
ETV5		*
FGFR1	*	
IGF2BP2		*
LPP		*
MAP3K13		*
MB21D2		*
MECOM		*
MUC4		*
PIK3CA		*
REL	*	
SOX2		*
TBL1XR1		*
TFRC		*
TP63		*
XPO1	*	

# Genes in Recurrence & LUSC – Primary III

Table: Deletion Genes in Recurrence & LUSC – Primary

CGC Genes	Non-recurrence	Recurrence
ACKR3	*	
ACSL3	*	
ACSL6	*	
AFF4	*	
APC	*	
ARHGPAP26	*	
ARHGEF10	*	
ATIC	*	
BARD1	*	
BCL2	*	
CASP8	*	
CD28	*	
CD74	*	
CDKN2A	*	*
COL3A1	*	
CREB1	*	
CSF1R	*	
CUL3	*	
DCC	*	
EBF1	*	
ERBB4	*	
FAT1	*	
FEV	*	
FGFR4	*	
FLT4	*	
HOXD11	*	
HOXD13	*	
IDH1	*	
ITGA7	*	
ITK	*	
KDSR	*	
MALT1	*	
NFE2L2	*	
NPM1	*	
NSD1	*	
PAX3	*	
PDGFRB	*	
PMS1	*	
PWWP2A	*	
SETBP1	*	
SF3B1	*	
SMAD2	*	
SMAD4	*	
TLX3	*	

# Findings in Recurrence & LUSC Gistic Results

## 4. Results

### 4.4. Copy Number Variation Analysis with Gistic

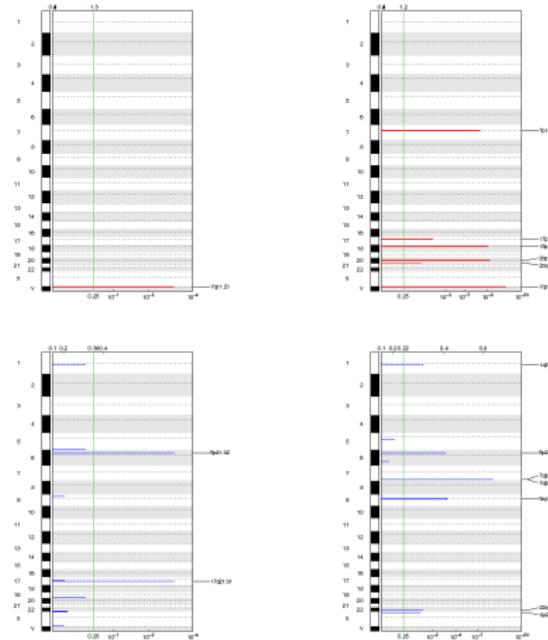
#### 4.4.4. Gistic in Recurrence & LUAD

# 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

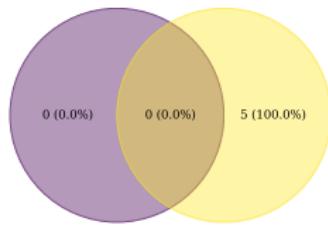
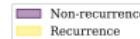
# Gistic in Recurrence & LUAD – AAH



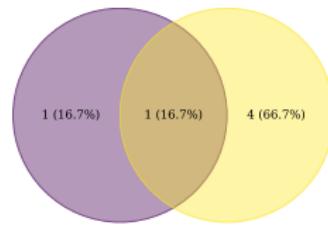
(a) Non-recurrence   (b) Recurrence

Figure: Gistic results in Recurrence & LUAD – AAH

# Peaks in Recurrence & LUAD – AAH I



(a) Amplification



(b) Deletion

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

# Peaks in Recurrence & LUAD – AAH II

Table: Amplification Peaks in Recurrence & LUAD – AAH

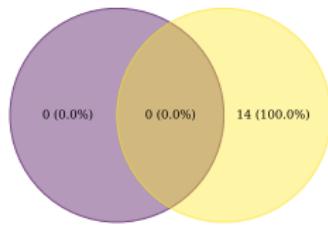
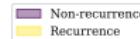
Peaks	Non-recurrence	Recurrence	CGC Genes
7p11.1		*	
17p11.1		*	
18p11.1		*	
20p11.1		*	
20q13.33		*	PTK6,SS18L1

# Peaks in Recurrence & LUAD – AAH III

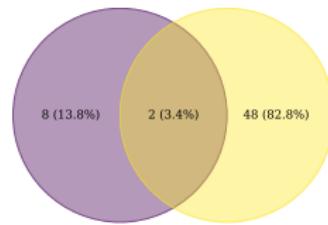
Table: Deletion Peaks in Recurrence & LUAD – AAH

Peaks	Non-recurrence	Recurrence	CGC Genes
1q21.3		*	ARNT,MLLT11,S100A7,SETDB1,TPM3
6p21.32	*	*	DAXX
7q35		*	CNTNAP2
9q13		*	
17q21.31	*		BRCA1,ETV4
22q13.1		*	APOBEC3B,MRTFA,PDGFB

# Genes in Recurrence & LUAD – AAH I



(a) Amplification



(b) Deletion

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

# Genes in Recurrence & LUAD – AAH II

Table: Amplification Genes in Recurrence & LUAD – AAH

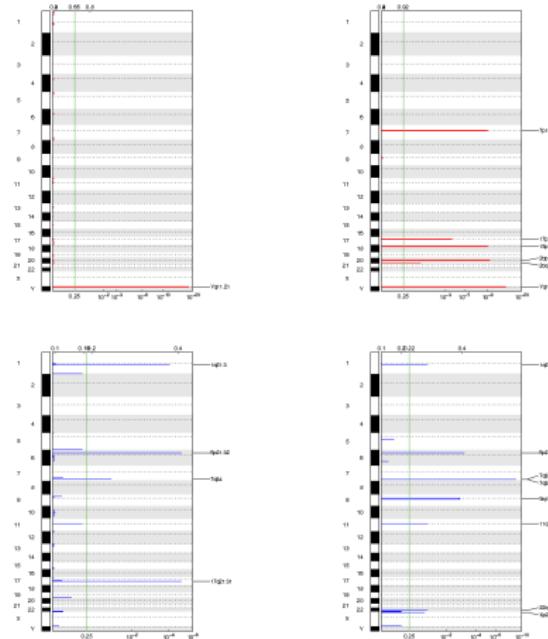
Genes	Non-recurrence	Recurrence
C20orf197		*
CDH26		*
CDH4		*
FRG1CP		*
LOC100506470		*
LOC101928048		*
LOC644669		*
LOC729296		*
MIR4533		*
MIR548AG2		*
MIR646		*
MIR646HG		*
MTRNR2L1		*
ZNF716		*

# Genes in Recurrence & LUAD – AAH III

Table: Deletion Genes in Recurrence & LUAD – AAH

Genes	Non-recurrence	Recurrence
ANKRD20A1	*	
ANKRD20A3	*	
ANKRD20A4	*	
APOBEC3A	*	
APOBEC3B-AS1	*	
AQP1P1	*	
AQP1P3	*	
AQP1P6P	*	
ARL11A	*	
ARL11B	*	
BTNL2	*	
Cblorf10		*
CBWD5		*
CTAGE4		*
CTAGE8		*
FAM27C		*
FAM74A1		*
FAM74A4		*
FAM98B1		*
FGF19P6		*
FLJ4315		*
FOOD44		*
FOOD45		*
FRG1LP		*
GXYLT1P3		*
HCG23		*
LCE3A		*
LCE3B		*
LINC01180		*
LINC01410		*
LOC100132249		*
LOC101920195		*
LOC101920193		*
LOC101920193		*
LOC101920983		*
LOC102723709		*
LOC102724238		*
LOC102724695		*
LOC38097		*
LOC403323		*
LOC554249		*
LOC562629		*
LOC562673		*
LRRK3TA		*
LRRK3TA2		*
MIR4427A		*
NSF	*	
NSF1	*	
OR2A1		*
OR2A2P		*
OR2A7		*
OR2A9P		*
PTENAMP2-CDK2AP2P2		*
SPATA11A5		*
SPATA11A7		*
XLOC_007897		*
ZNF658		*

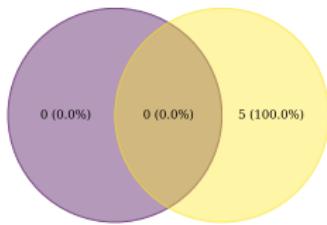
# Gistic in Recurrence & LUAD – Precancer



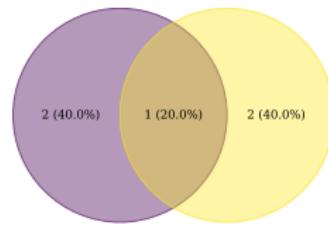
(a) Non-recurrence    (b) Recurrence

Figure: Gistic results in Recurrence & LUAD – Precancer

# Peaks in Recurrence & LUAD – Precancer I



(a) Amplification



(b) Deletion

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

# Peaks in Recurrence & LUAD – Precancer II

Table: Amplification Peaks in Recurrence & LUAD – Precancer

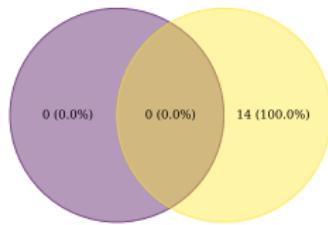
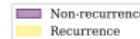
Peaks	Non-recurrence	Recurrence	CGC Genes
7p11.1		*	
17p11.1		*	
18p11.1		*	
20p11.1		*	
20q13.33		*	PTK6,SS18L1

# Peaks in Recurrence & LUAD – Precancer III

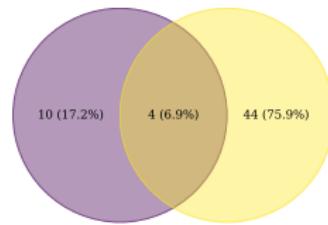
Table: Deletion Peaks in Recurrence & LUAD – Precancer

Peaks	Non-recurrence	Recurrence	CGC Genes
1q21.3	*	*	ARNT,MLLT11,S100A7,SETDB1,TPM3
6p21.32	*	*	DAXX
7q34	*		BRAF,FAM131B,KIAA1549,TRIM24
7q35		*	CNTNAP2
9q13		*	
11q11		*	
17q21.31	*		BRCA1,ETV4
22q13.1		*	APOBEC3B,MRTFA,PDGFB

# Genes in Recurrence & LUAD – Precancer I



(a) Amplification



(b) Deletion

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

# Genes in Recurrence & LUAD – Precancer II

Table: Amplification Genes in Recurrence & LUAD – Precancer

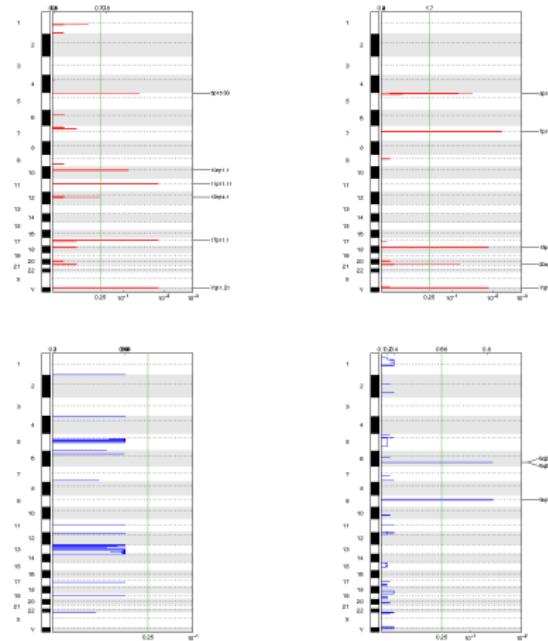
Genes	Non-recurrence	Recurrence
C20orf197		*
CDH26		*
CDH4		*
FRG1CP		*
LOC100506470		*
LOC101928048		*
LOC644669		*
LOC729296		*
MIR4533		*
MIR548AG2		*
MIR646		*
MIR646HG		*
MTRNR2L1		*
ZNF716		*

Genes in Recurrence & LUAD – Precancer III

**Table: Deletion Genes in Recurrence & LUAD – Precancer**

	Non-recurrence	Recurrence
Gems		
ANKR020A1	*	
ANKR020A3	*	
ANKR020A4	*	
ANRHEF1P1	*	
AQP1P1	*	
ARHGEF34P	*	
ARL17A	*	
ARL1B	*	
C12orf10	*	
CBWD5		
CTAGE4		
CTAGE8		
FAM102C		
FAM14A1		
FAM14A3		
FAM14A4		
FAM14A5		
FAM14A6		
FGF19p		
FLJ43315		
FOXA4L		
FOXP2A5		
GAPDHP		
GXYLT1P3		
HCG23	*	*
LCE3A	*	
LCE3B	*	
LCE3C	*	
LCE3D	*	
LCE3E	*	
LINC01189		
LINC01194		
LOC_100132349		
LOC_101020395		
LOC_101028381		
LOC_101029163	*	
LOC_101029483		
LOC_105723509		
LOC_105724338		
LOC_105908605		
LOC_286597		
LOC_352323		
LOC_354249		
LOC_424629		
LOC_728673		
LRRC47A		
LRRC57A2	*	
MIR447A		
NSFP1	*	
OR41		
OR42A3P		
OR47A		
OR42A9P		
PTGER4P5-CDK2AP2P2		
SATB2A1A		
SATP1A1A		
XLOC_007687		
ZNF658		

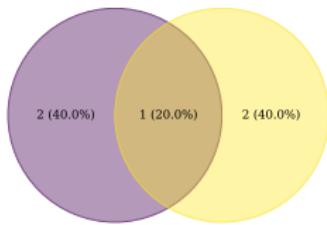
# Gistic in Recurrence & LUAD – Primary



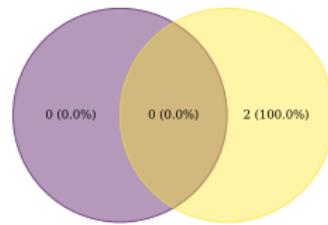
(a) Non-recurrence   (b) Recurrence

Figure: Gistic results in Recurrence & LUAD – Primary

# Peaks in Recurrence & LUAD – Primary I



(a) Amplification



(b) Deletion

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

# Peaks in Recurrence & LUAD – Primary II

Table: Amplification Peaks in Recurrence & LUAD – Primary

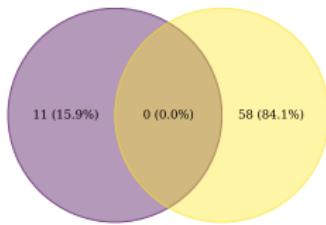
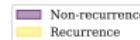
Peaks	Non-recurrence	Recurrence	CGC Genes
5p15.33	*	*	SDHA, TERT
7p11.1		*	
10q11.1	*		
11p11.11	*		
12q14.1	*		CDK4, LRIG3
17p11.1	*		
18p11.1		*	
20q13.33		*	PTK6, SS18L1

# Peaks in Recurrence & LUAD – Primary III

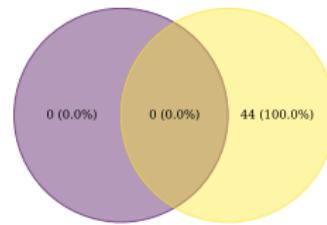
Table: Deletion Peaks in Recurrence & LUAD – Primary

Non-recurrence Peaks	Recurrence	CGC Genes
6q22.31	*	
9q13	*	

# Genes in Recurrence & LUAD – Primary I



(a) Amplification



(b) Deletion

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

# Genes in Recurrence & LUAD – Primary II

Table: Amplification Genes in Recurrence & LUAD – Primary

CGC Genes	Non-recurrence	Recurrence
CTNND2		*
TERT	*	

# Genes in Recurrence & LUAD – Primary III

Table: Deletion Genes in Recurrence & LUAD – Primary

Genes	Non-recurrence	Recurrence
ANKRD20A1	*	
ANKRD20A3	*	
ANKRD20A4	*	
AQP7P1	*	
AQP7P3	*	
CBWD5	*	
CNTNAP3P2	*	
FAM27B	*	
FAM27C	*	
FAM27E3	*	
FAM74A1	*	
FAM74A3	*	
FAM74A4	*	
FAM95B1	*	
FGF7P6	*	
FLJ43315	*	
FOXD4L4	*	
FOXD4L5	*	
FRG1JP	*	
GXYLT1P3	*	
HRAT13	*	
LINC01189	*	
LINC01410	*	
LOC100132249	*	
LOC101928195	*	
LOC101928381	*	
LOC101929583	*	
LOC102723709	*	
LOC102724238	*	
LOC103900865	*	
LOC105379450	*	
LOC286297	*	
LOC403323	*	
LOC554249	*	
LOC642929	*	
LOC728673	*	
MIR4477A	*	
PTGER4P2-CDK2AP2P2	*	
SPATA31A3	*	
SPATA31A5	*	
SPATA31A7	*	
TRDN	*	
XLOC_007697	*	
ZNF658	*	

# Findings in Recurrence & LUAD Gistic Results

## 4. Results

### 4.4. Copy Number Variation Analysis with Gistic

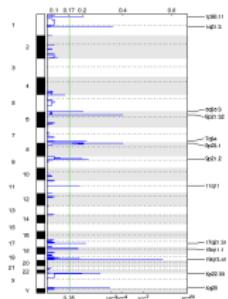
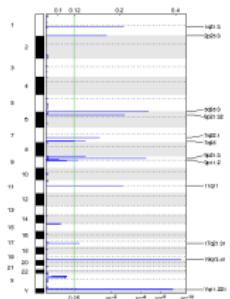
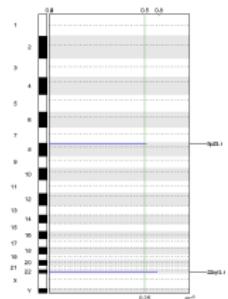
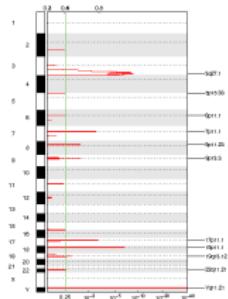
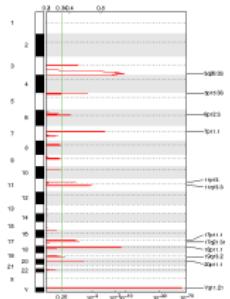
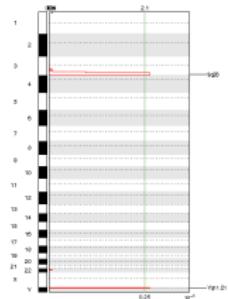
#### 4.4.5. Gistic in Smoking & LUSC

# 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	

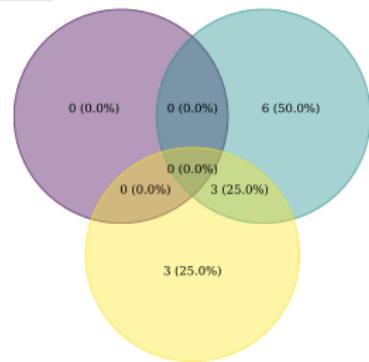
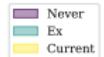
# Gistic in Smoking & LUSC – CIS



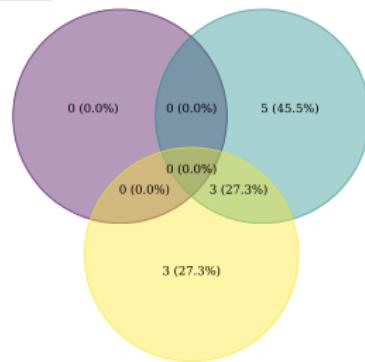
(a) Never Smoker   (b) Ex-Smoker   (c) Current Smoker

Figure: Gistic results in Smoking & LUSC – CIS

# Peaks in Smoking & LUSC – CIS I



(a) Amplification



(b) Deletion

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

# Peaks in Smoking & LUSC – CIS II

Table: Amplification Peaks in Smoking & LUSC – CIS

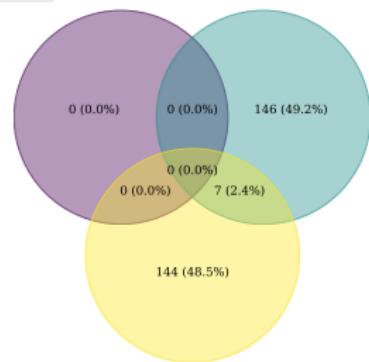
Peaks	Never	Ex	Current	CGC Genes
3q26.33		*		SOX2
3q27.1			*	
3q28	*			LPP,TP63
5p15.33		*	*	SDHA,TERT
6p11.1			*	
6p12.3		*		
7p11.1		*	*	
8p11.23			*	FGFR1,NSD3
9p13.3			*	FANCG
11p13		*		LMO2,WT1
11q13.3		*		CCND1
17p11.1		*	*	
17q21.31		*		BRCA1,ETV4
18p11.1		*	*	
19q13.2		*		AKT2,CD79A,CIC
19q13.12			*	
20p11.1		*		
22q11.21		*		CLTCL1,DGCR8,LZTR1,SEPT5

# Peaks in Smoking & LUSC – CIS III

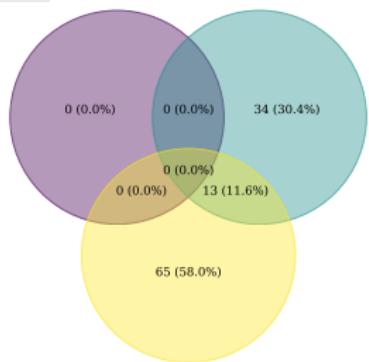
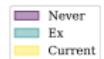
Table: Deletion Peaks in Smoking & LUSC – CIS

Peaks	Never	Ex	Current	CGC Genes
1p36.11			*	ARID1A,MDS2
1q21.3		*	*	ARNT,MLLT11,S100A7,SETDB1,TPM3
2p25.3		*		
5q35.3		*	*	FLT4,NSD1
6p21.32		*	*	DAXX
7q22.1		*		CUX1,TRRAP
7q34			*	BRAF,FAM131B,KIAA1549,TRIM24
7q35		*		CNTNAP2
8p23.1	*		*	
9p11.2		*		
9p21.2			*	
9p21.3		*		CDKN2A,MLLT3
11q11		*	*	
17q21.31		*	*	BRCA1,ETV4
18q11.1			*	
19q13.41		*	*	PPP2R1A
22q13.1	*			APOBEC3B,MRTFA,PDGFB

# Genes in Smoking & LUSC – CIS I



(a) Amplification



(b) Deletion

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

# Genes in Smoking & LUSC – CIS II

Table: Amplification Genes in Smoking & LUSC – CIS

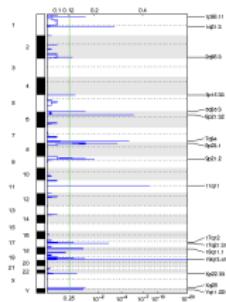
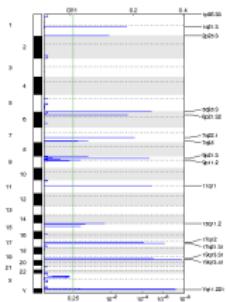
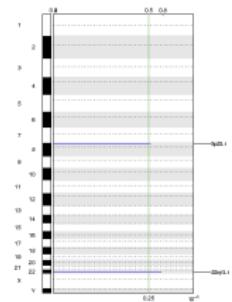
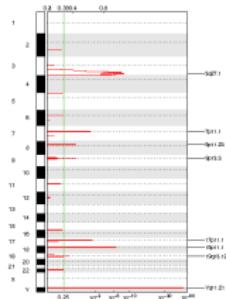
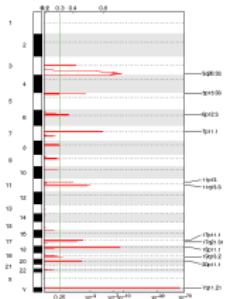
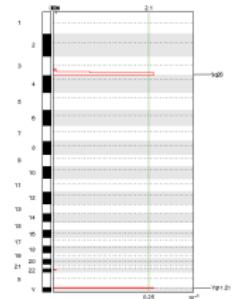
CGC Genes	Never	Ex	Current
ANK1			*
CCND1		*	
EXT2		*	
FANCG			*
FGFR1			*
HOOK3			*
IKBKB			*
KAT6A			*
LMO2		*	
PAX5			*
SOX2		*	
WT1		*	

# Genes in Smoking & LUSC – CIS III

Table: Deletion Genes in Smoking & LUSC – CIS

CGC Genes	Never	Ex	Current
CDKN2A		*	*
MLLT3			*

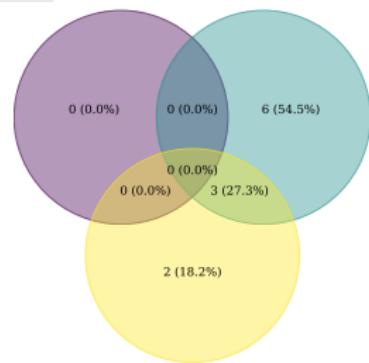
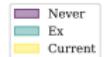
# Gistic in Smoking & LUSC – Precancer



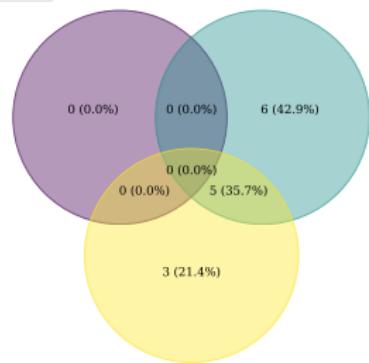
(a) Never Smoker   (b) Ex-Smoker   (c) Current Smoker

Figure: Gistic results in Smoking & LUSC – Precancer

# Peaks in Smoking & LUSC – Precancer I



(a) Amplification



(b) Deletion

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

# Peaks in Smoking & LUSC – Precancer II

Table: Amplification Peaks in Smoking & LUSC – Precancer

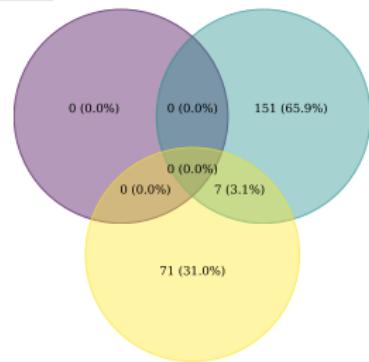
Peaks	Never	Ex	Current	CGC Genes
3q26.33		*		SOX2
3q27.1			*	
3q28	*			LPP,TP63
5p15.33		*		SDHA,TERT
6p12.3		*		
7p11.1	*		*	
8p11.23			*	FGFR1,NSD3
9p13.3			*	FANCG
11p13		*		LMO2,WT1
11q13.3		*		CCND1
17p11.1	*		*	
17q21.31		*		BRCA1,ETV4
18p11.1	*		*	
19q13.2	*			AKT2,CD79A,CIC
19q13.12			*	
20p11.1		*		

# Peaks in Smoking & LUSC – Precancer III

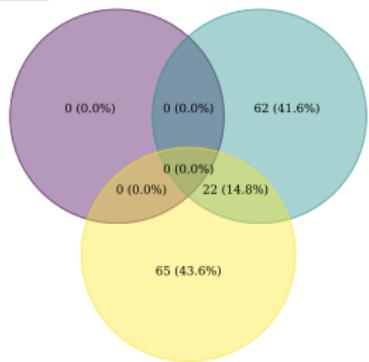
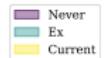
Table: Deletion Peaks in Smoking & LUSC – Precancer

Peaks	Never	Ex	Current	CGC Genes
1p36.11			*	ARID1A,MDS2
1p36.33		*		SKI
1q21.3	*	*		ARNT,MLLT11,S100A7,SETDB1,TPM3
2p25.3	*			
2q36.3		*		
5p15.33		*		SDHA,TERT
5q35.3	*	*		FLT4,NSD1
6p21.32	*	*		DAXX
7q22.1	*			CUX1,TRRAP
7q34		*		BRAF,FAM131B,KIAA1549,TRIM24
7q35	*			CNTNAP2
8p23.1	*	*		
9p11.2		*		
9p21.2		*		
9p21.3	*			CDKN2A,MLLT3
11q11	*	*		
15q11.2	*			
17q12	*	*		CDK12,ERBB2,LASP1,MLLT6,TAF15
17q21.31	*	*		BRCA1,ETV4
18q11.1		*		
19q13.31	*			
19q13.41	*	*		PPP2R1A
22q13.1	*			APOBEC3B,MRTFA,PDGFB

# Genes in Smoking & LUSC – Precancer I



(a) Amplification



(b) Deletion

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

# Genes in Smoking & LUSC – Precancer II

Table: Amplification Genes in Smoking & LUSC – Precancer

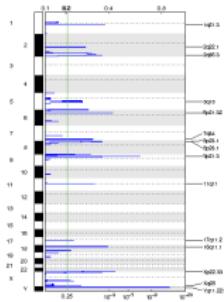
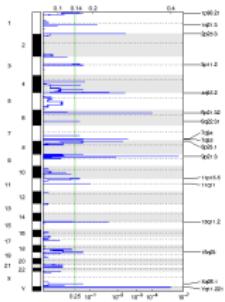
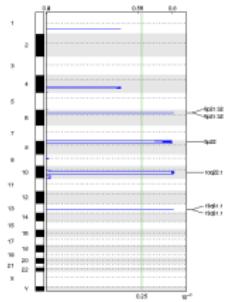
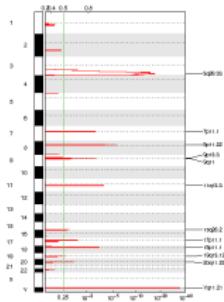
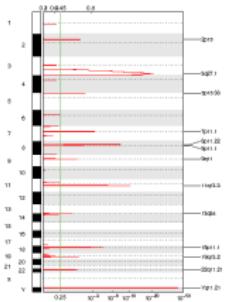
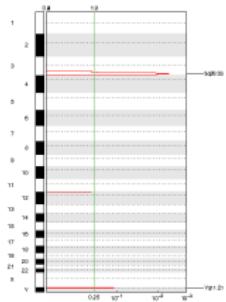
CGC Genes	Never	Ex	Current
CCND1		*	
EXT2		*	
FANCG			*
LMO2		*	
PAX5			*
SOX2		*	
WT1		*	

# Genes in Smoking & LUSC – Precancer III

Table: Deletion Genes in Smoking & LUSC – Precancer

CGC Genes	Never	Ex	Current
CDKN2A		*	*
MLLT3			*

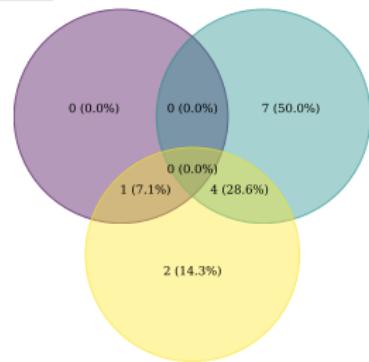
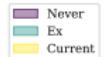
# Gistic in Smoking & LUSC – Primary



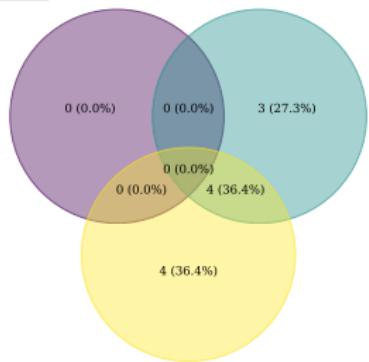
(a) Never Smoker   (b) Ex-Smoker   (c) Current Smoker

Figure: Gistic results in Smoking & LUSC – Primary

# Peaks in Smoking & LUSC – Primary I



(a) Amplification



(b) Deletion

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

# Peaks in Smoking & LUSC – Primary II

Table: Amplification Peaks in Smoking & LUSC – Primary

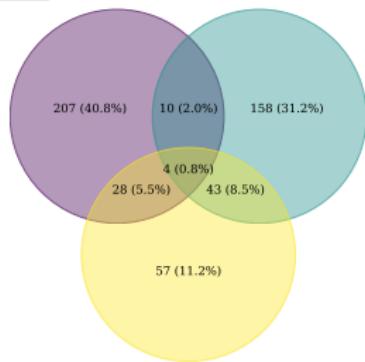
Peaks	Never	Ex	Current	CGC Genes
2p15		*		XPO1
3q26.33	*		*	SOX2
3q27.1		*		
5p15.33		*		SDHA,TERT
7p11.1		*	*	
8p11.1		*		
8p11.22		*	*	
9p13.3			*	FANCG
9q11		*	*	
11q13.3		*	*	CCND1
13q34		*		
15q26.2			*	
17p11.1			*	
18p11.1		*	*	
19q13.2		*		AKT2,CD79A,CIC
19q13.12			*	
20q11.22			*	
22q11.21		*		CLTCL1,DGCR8,LZTR1,SEPT5

# Peaks in Smoking & LUSC – Primary III

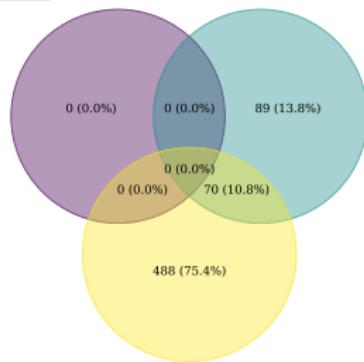
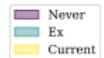
Table: Deletion Peaks in Smoking & LUSC – Primary

Peaks	Never	Ex	Current	CGC Genes
1p36.21		*		CASP9, PRDM2, SPEN
1q21.3		*	*	ARNT, MLLT11, S100A7, SETDB1, TPM3
2p25.3		*		
2q22.1			*	CXCR4, LRP1B
2q36.3			*	
3p11.2		*		
4q35.2		*		DUX4L1, FAT1
5q15			*	
6p21.32	*	*	*	DAXX
6q22.31		*		
7q34		*	*	BRAF, FAM131B, KIAA1549, TRIM24
7q35		*		CNTNAP2
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		*		

# Genes in Smoking & LUSC – Primary I



(a) Amplification



(b) Deletion

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

# Genes in Smoking & LUSC – Primary II

Table: Amplification Genes in Smoking & LUSC – Primary

CGC Genes	Never	Ex	Current
AKT2	*		
ANK1			*
BCL11A		*	
BCL6	*		
CCND1			*
DGCR8		*	
EIF4A2	*		
ETV5	*		
FGFR1		*	*
HOOK3			*
IGF2BP2	*		
IKBKB			*
KAT6A			*
LPP	*		
LZTR1		*	
MAP3K13	*		
MB21D2	*		
MUC4	*		
PIK3CA	*		*
REL		*	
SOX2	*	*	*
TBL1XR1	*		
TFRC	*		
TP63	*		
XPO1		*	

# Genes in Smoking & LUSC – Primary III

Table: Deletion Genes in Smoking & LUSC – Primary

CGC Genes	Never	Ex	Current
ACKR3			*
ACSL3			*
ARHGEF10	*	*	
ATIC			*
BARD1			*
CASP3		*	
CDKN2A	*	*	
CREB1			*
CUL3			*
ERBB4			*
FAT1	*		
FEV			*
IDH1			*
PAX3			*

# Findings in Smoking & LUSC Gistic Results

## 4. Results

### 4.4. Copy Number Variation Analysis with Gistic

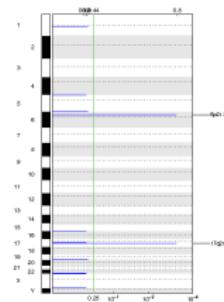
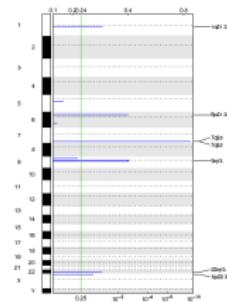
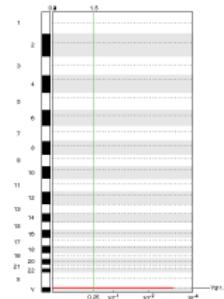
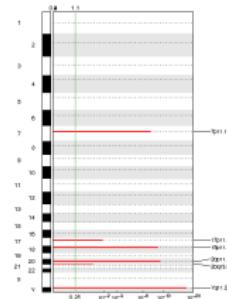
#### 4.4.6. Gistic in Smoking & LUAD

# 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	

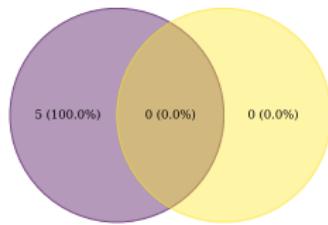
# Gistic in Smoking & LUAD – AAH



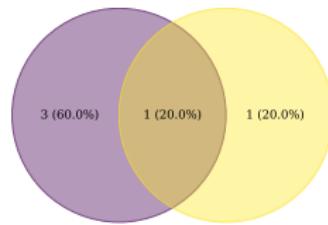
(a) Ex-Smoker   (b) Current Smoker

Figure: Gistic results in Smoking & LUAD – AAH

# Peaks in Smoking & LUAD – AAH I



(a) Amplification



(b) Deletion

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

# Peaks in Smoking & LUAD – AAH II

Table: Amplification Peaks in Smoking & LUAD – AAH

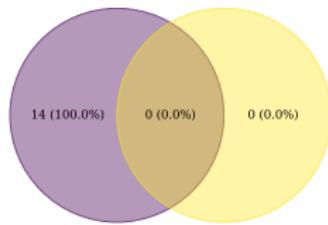
Peaks	Ex	Current	CGC Genes
7p11.1	*		
17p11.1	*		
18p11.1	*		
20p11.1	*		
20q13.33	*		PTK6,SS18L1

# Peaks in Smoking & LUAD – AAH III

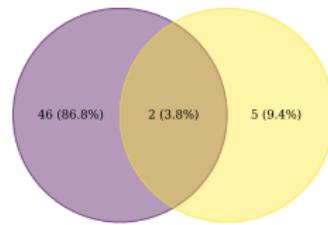
Table: Deletion Peaks in Smoking & LUAD – AAH

Peaks	Ex	Current	CGC Genes
1q21.3	*		ARNT,MLLT11,S100A7,SETDB1,TPM3
6p21.32	*	*	DAXX
7q35	*		CNTNAP2
9q13	*		
17q21.31		*	BRCA1,ETV4
22q13.1	*		APOBEC3B,MRTFA,PDGFB

# Genes in Smoking & LUAD – AAH I



(a) Amplification



(b) Deletion

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

# Genes in Smoking & LUAD – AAH II

Table: Amplification Genes in Smoking & LUAD – AAH

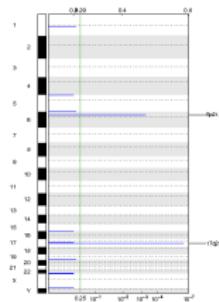
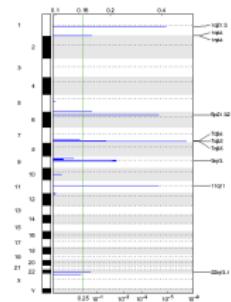
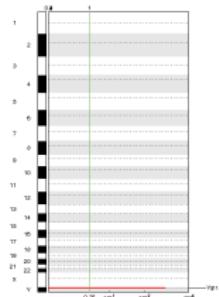
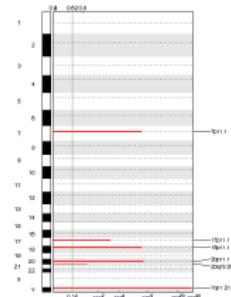
Genes	Ex	Current
C20orf197	*	
CDH26	*	
CDH4	*	
FRG1CP	*	
LOC100506470	*	
LOC101928048	*	
LOC644669	*	
LOC729296	*	
MIR4533	*	
MIR548AG2	*	
MIR646	*	
MIR646HG	*	
MTRNR2L1	*	
ZNF716	*	

# Genes in Smoking & LUAD – AAH III

Table: Deletion Genes in Smoking & LUAD – AAH

Genes	Ex	Current
ANKRD20A1	*	
ANKRD20A3	*	
ANKRD20A4	*	
AQP7P1	*	
AQP7P3	*	
ARHGEF34P	*	
ARL17A	*	*
ARL17B	*	*
Cebpd10		*
CEVND5		*
CTAGE4		*
CTAGE8		*
FAM27C		*
FAM74A1		*
FAM74A3		*
FAM74A4		*
FAM19A3B		*
FGF7P6		*
FLJ43315		*
FOXD4L4		*
FOXD4L5		*
FRG1JP		*
GKYL1P1P		*
LCE3A		*
LINC01189		*
LINC01410		*
LOC100132249		*
LOC101928195		*
LOC101928388		*
LOC101929163		*
LOC101929583		*
LOC101929709		*
LOC101929728		*
LOC1019309605		*
LOC286297		*
LOC403323		*
LOC554249		*
LOC642929		*
LOC728673		*
LRRC37A		*
LRRC37A2		*
MIR4477A		*
NSFP1		*
OR2A1		*
OR2A20P		*
OR2A7		*
OR2A9P		*
PTENAMP3-CDK2AP2P2		*
SPATA31A5		*
SPATA31A7		*
XLOC_007607		*
ZNF658		*

# Gistic in Smoking & LUAD – Precancer

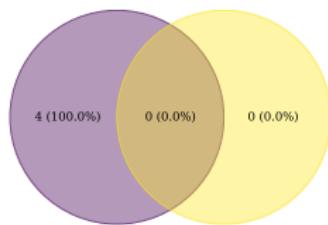


(a) Ex-Smoker

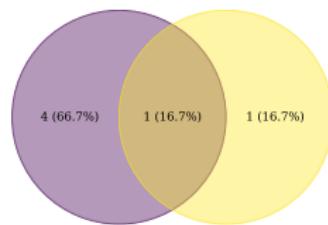
(b) Current Smoker

Figure: Gistic results in Smoking & LUAD – Precancer

# Peaks in Smoking & LUAD – Precancer I



(a) Amplification



(b) Deletion

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

# Peaks in Smoking & LUAD – Precancer II

Table: Amplification Peaks in Smoking & LUAD – Precancer

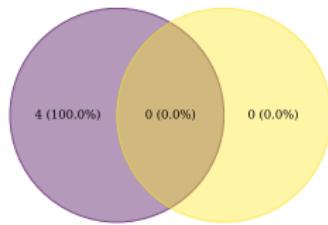
Peaks	Ex	Current	CGC Genes
7p11.1	*		
17p11.1	*		
18p11.1	*		
20p11.1	*		
20q13.33	*		PTK6,SS18L1

# Peaks in Smoking & LUAD – Precancer III

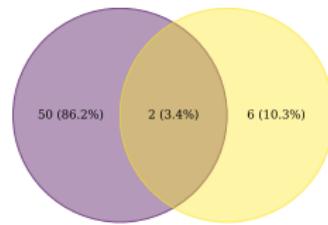
Table: Deletion Peaks in Smoking & LUAD – Precancer

Peaks	Ex	Current	CGC Genes
1q21.3	*		ARNT,MLLT11,S100A7,SETDB1,TPM3
1q44	*		AKT3
6p21.32	*	*	DAXX
7q34	*		BRAF,FAM131B,KIAA1549,TRIM24
7q35	*		CNTNAP2
9q13	*		
11q11	*		
17q21.31		*	BRCA1,ETV4
22q13.1	*		APOBEC3B,MRTFA,PDGFB

# Genes in Smoking & LUAD – Precancer I



(a) Amplification



(b) Deletion

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

# Genes in Smoking & LUAD – Precancer II

Table: Amplification Genes in Smoking & LUAD – Precancer

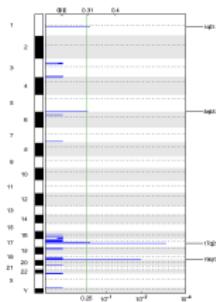
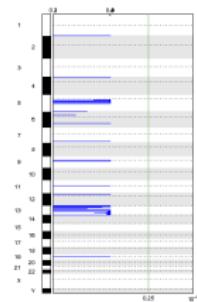
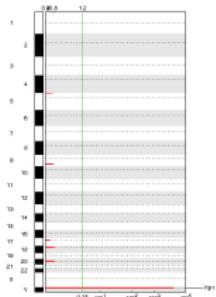
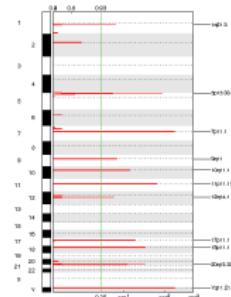
Genes	Ex	Current
FRG1CP	*	
LOC644669	*	
MTRNR2L1	*	
ZNF716	*	

# Genes in Smoking & LUAD – Precancer III

Table: Deletion Genes in Smoking & LUAD – Precancer

Genes	Ex	Current
ANKRD26A1	*	
ANKRD26A3	*	
ANKRD26A4	*	
AQP9P1		
AGRP7P9		
ARHGEF3AP	*	
ARL17A		*
ARL17B		*
Ctorf10	*	
CBWD5		
CTAGE4		
CTAGE8		
FAM27C		
FAM74A1		
FAM74A3		
FAM74A4		
FAM98B1		
FGF7P6		
FLJ43515		
FOOD44		
FOXO4A5		
FRG1LP		
GXYLT1P3		
HCG23		
LCE3A		
LCE3B		
LINC01189		
LINC01410		
LOC100132249		
LOC101981995		
LOC101981996		
LOC101981983		
LOC101989883		
LOC102723709		
LOC102742420		
LOC103980005		
LOC26027		
LOC403323		
LOC594249		
LOC642029		
LOC729972		
LRRK3TA		
LRRK3TA2		
MIR4477A		
NSFP1		
O84241		
O84246P		
O84247		
O84248P		
O84C11		
O84E5		
O84H4		
O84S2		
PTGER4P2-CDK2AP2P2	*	
SPATA31A5		
SPATA31A7		
XLOC_097647		
ZNF658	*	

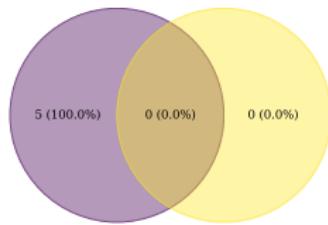
# Gistic in Smoking & LUAD – Primary



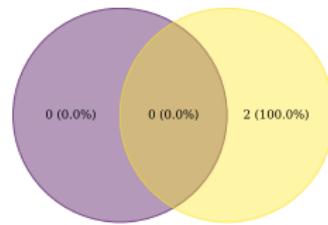
(a) Ex-Smoker   (b) Current Smoker

Figure: Gistic results in Smoking & LUAD – Primary

# Peaks in Smoking & LUAD – Primary I



(a) Amplification



(b) Deletion

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

# Peaks in Smoking & LUAD – Primary II

Table: Amplification Peaks in Smoking & LUAD – Primary

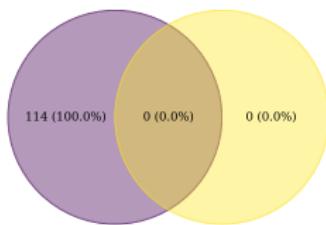
Peaks	Ex	Current	CGC Genes
1q21.3	*		ARNT,MLLT11,S100A7,SETDB1,TPM3
5p15.33	*		SDHA,TERT
7p11.1	*		
9q11	*		
10q11.1	*		
11p11.11	*		
12q14.1	*		CDK4,LRIG3
17p11.1	*		
18p11.1	*		
20q13.33	*		PTK6,SS18L1

# Peaks in Smoking & LUAD – Primary III

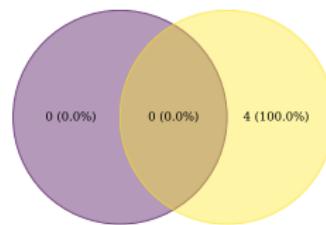
Table: Deletion Peaks in Smoking & LUAD – Primary

Ex Peaks	Current	CGC Genes
1q21.3	*	ARNT,MLLT11,S100A7,SETDB1,TPM3
5q35.3	*	FLT4,NSD1
17q21.31	*	BRCA1,ETV4
19q13.42	*	CNOT3,TFPT,ZNF331

# Genes in Smoking & LUAD – Primary I



(a) Amplification



(b) Deletion

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

# Genes in Smoking & LUAD – Primary II

Table: Amplification Genes in Smoking & LUAD – Primary

CGC Genes	Ex	Current
PTK6	*	
SS18L1	*	

# Genes in Smoking & LUAD – Primary III

Table: Deletion Genes in Smoking & LUAD – Primary

Genes	Ex	Current
ARL17A		*
ARL17B		*
LILRB4		*
MIR8061		*

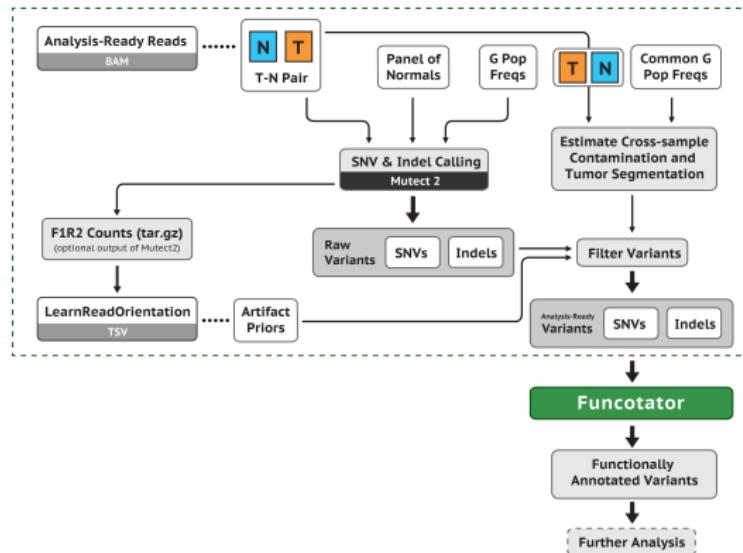
# Findings in Smoking & LUAD Gistic Results

# Findings in Gistic

## 4. Results

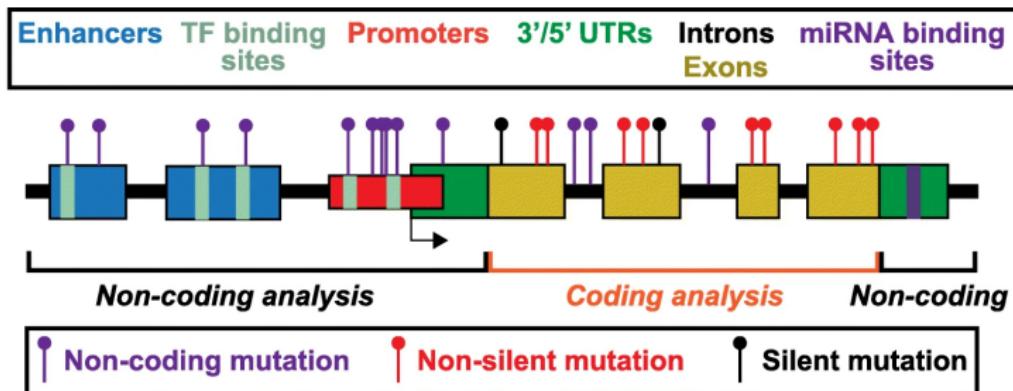
### 4.5. Single Nucleotide Variations Analysis

# 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?

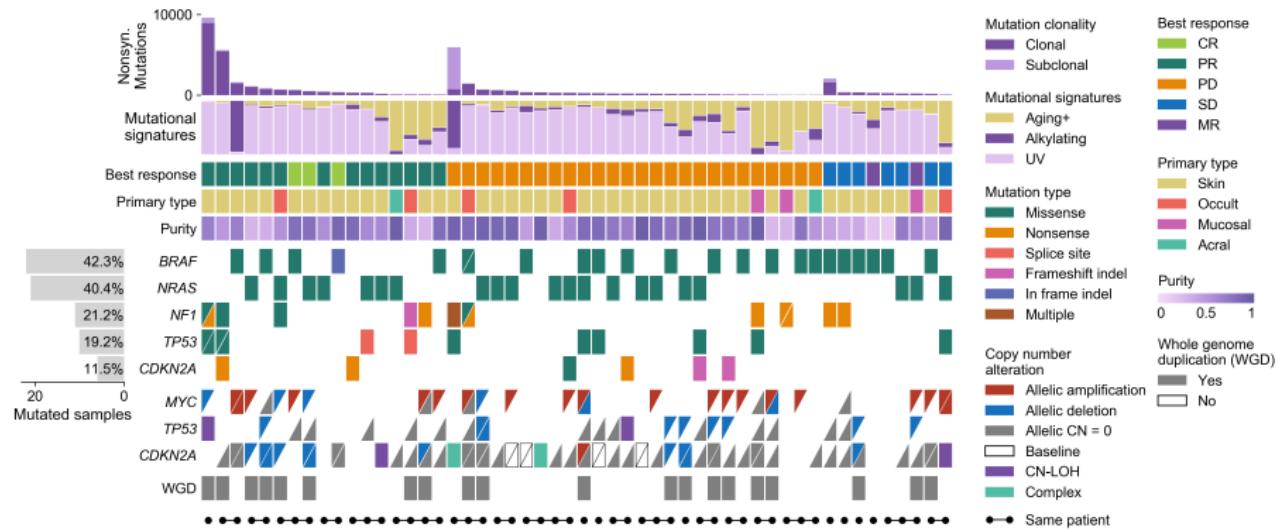


Figure: A comutation plot generated with CoMut (Crowdis et al., 2020)

# 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

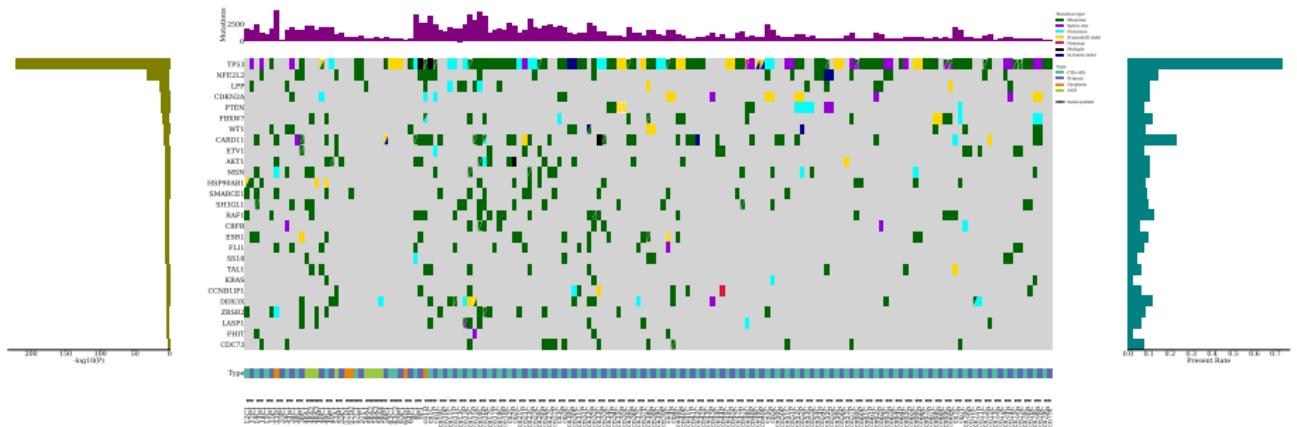


Figure: CoMut Plot with LUSC Patients

# Somatic Variant in LUSC with Recurrence

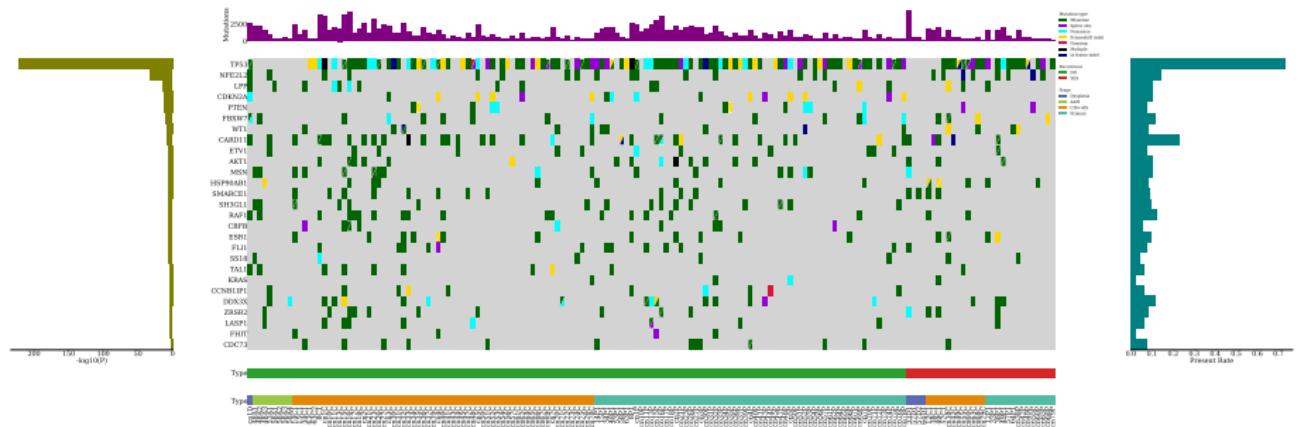


Figure: CoMut Plot in LUSC Patients with Recurrence

# Somatic Variant in LUAD

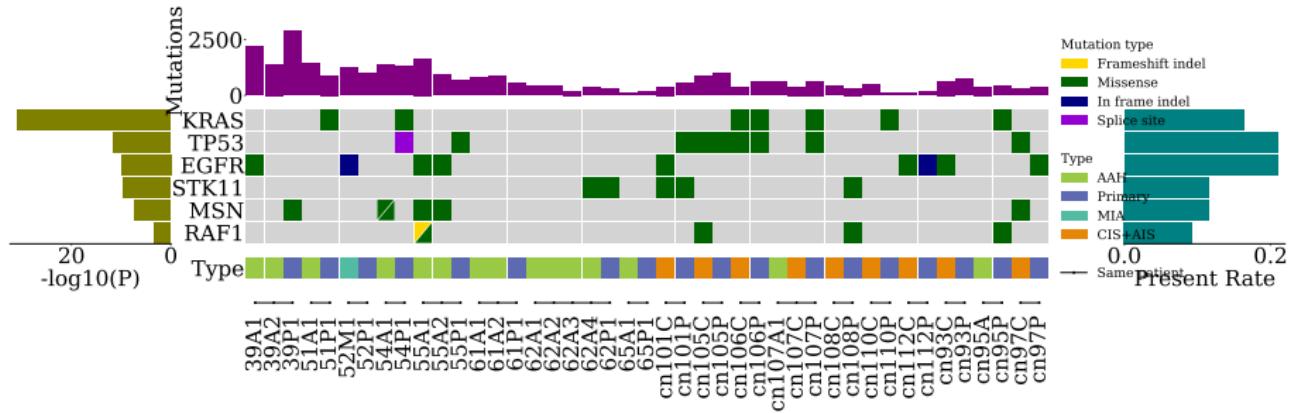


Figure: CoMut Plot with LUAD Patients

# Somatic Variant in LUAD with Recurrence

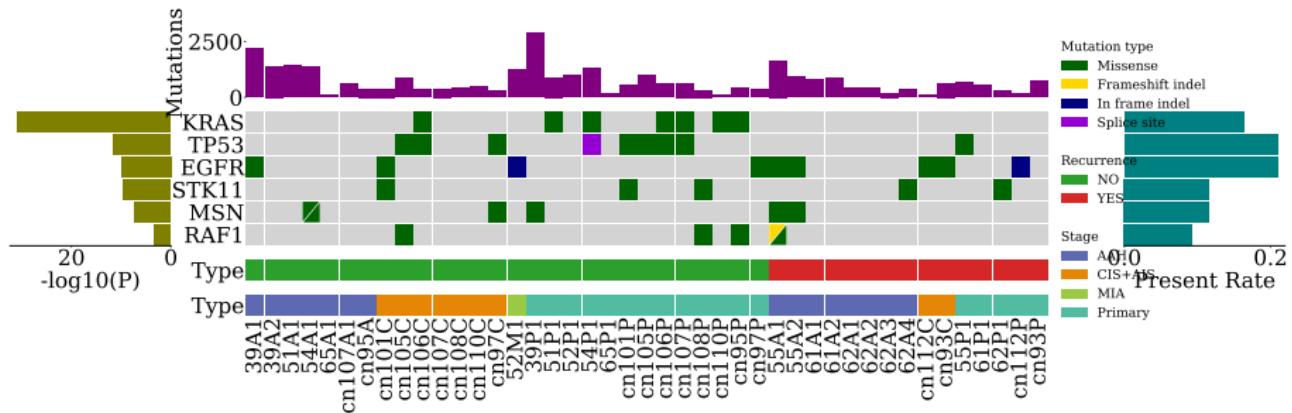


Figure: CoMut Plot in LUAD Patients with Recurrence

# Findings in SNVs Analysis

## 4. Results

### 4.6. VAF Analysis

# VAF?

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

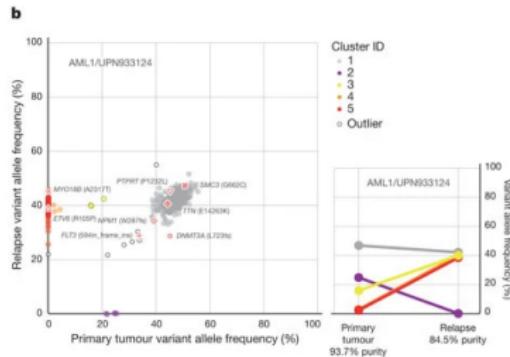
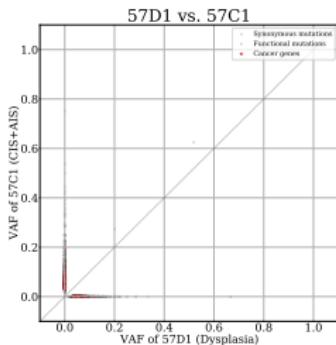
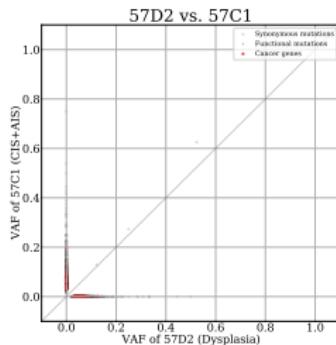


Figure: VAF distribution of validated mutations (L. Ding et al., 2012)

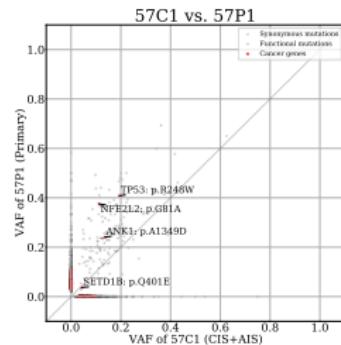
# VAF Plots



(a) Dysplasia + CIS



(b) Dysplasia + CIS



(c) CIS + Primary

Figure: VAF plots in patient #57

# PyClone?

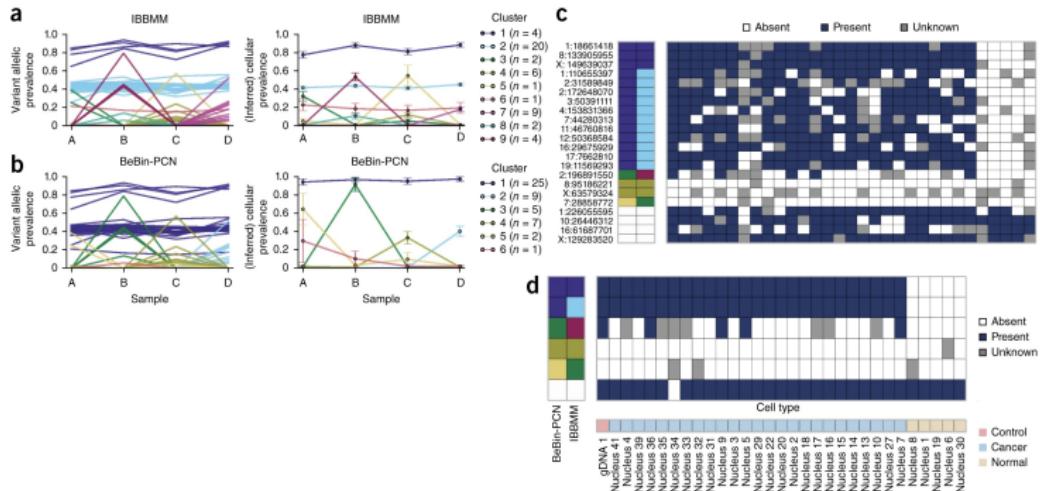
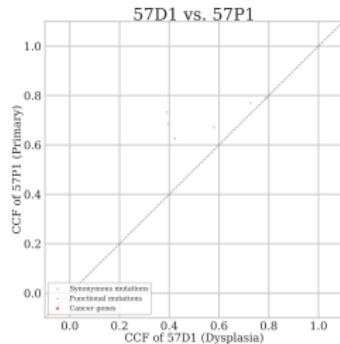
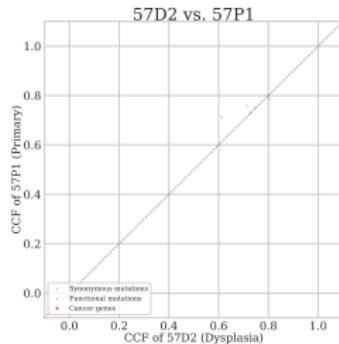


Figure: Analysis of multiple samples by PyClone (Roth et al., 2014)

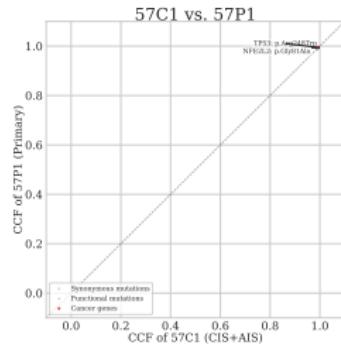
# PyClone Plots I



(a) 57D1



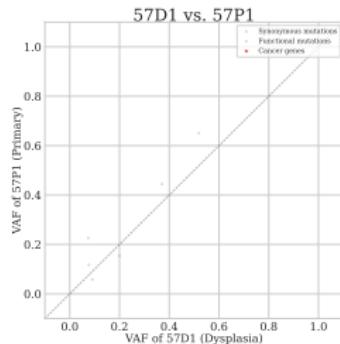
(b) 57D2



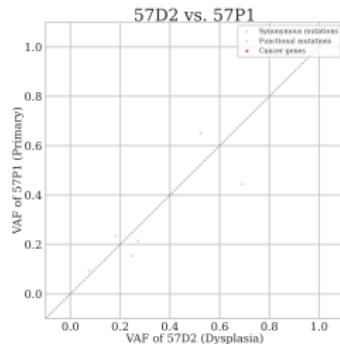
(c) 57C1

Figure: CCF plot in patient #57

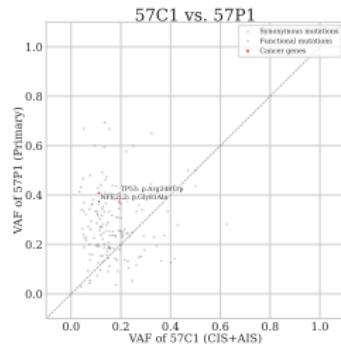
# PyClone Plots II



(a) 57D1



(b) 57D2



(c) 57C1

Figure: VAF plot in patient #57

# Findings in VAF Analysis

## 4. Results

### 4.7. Tumor Evolution Trajectories Analysis

# Mobster?

# Findings in Tumor Evolution Trajectories Analysis

## 4. Results

### 4.8. Bulk Cell Deconvolution

# BisqueRNA?

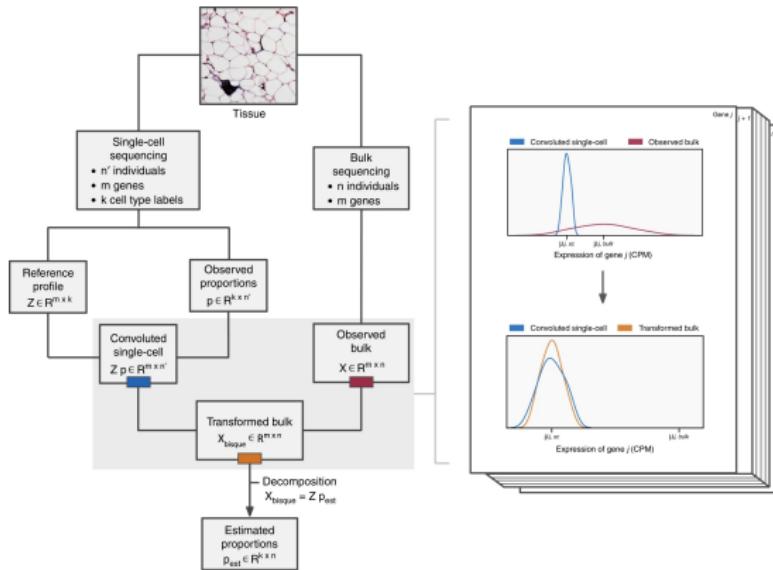


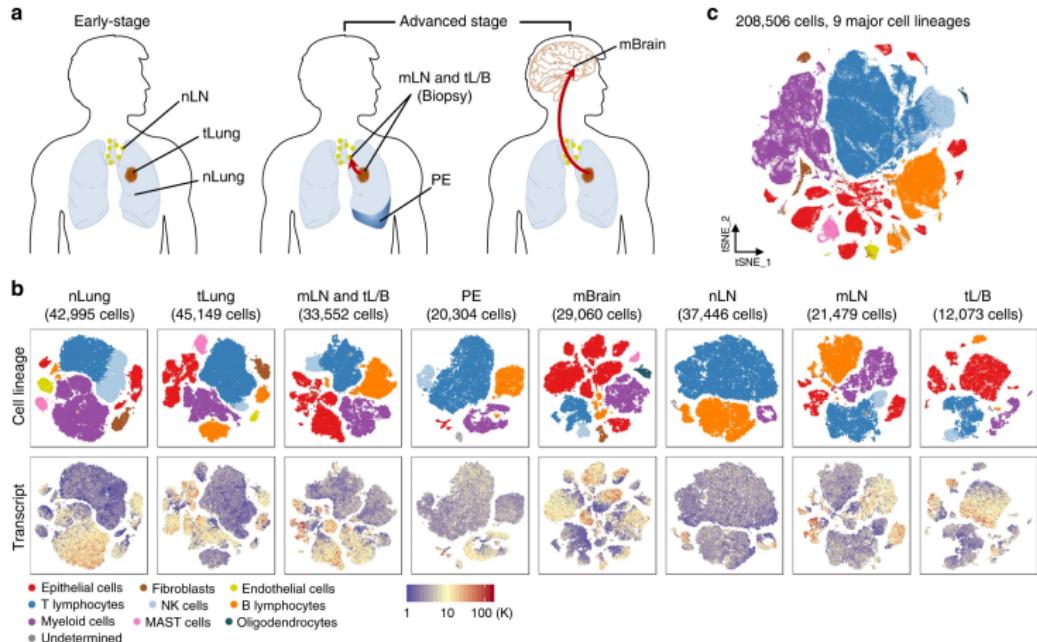
Figure: Workflow for BisqueRNA (Jew et al., 2020)

## 4. Results

### 4.8. Bulk Cell Deconvolution

#### 4.8.1. Reference by 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

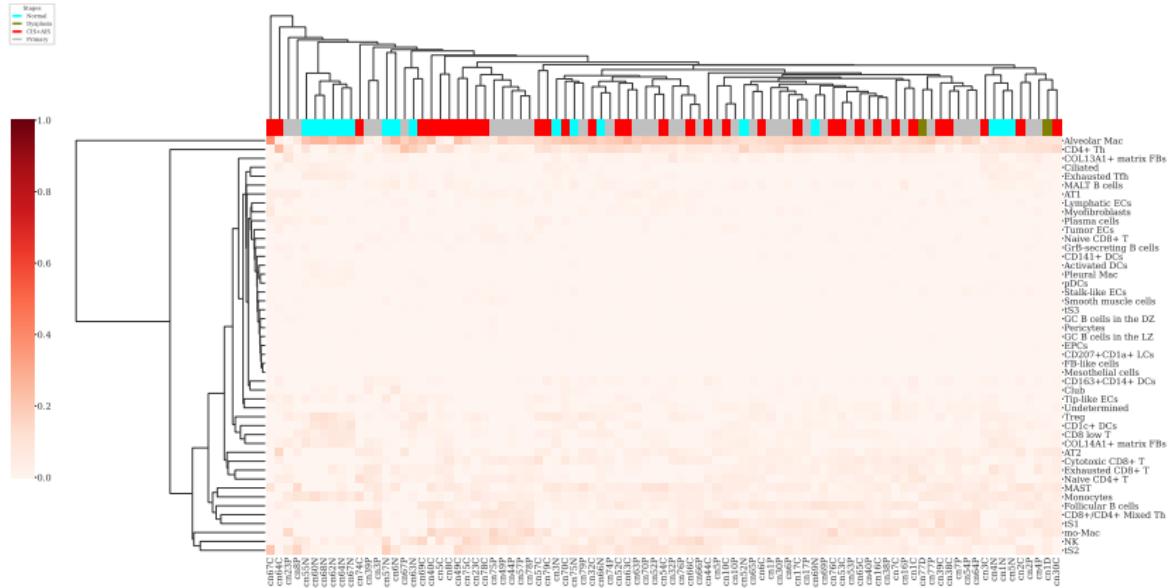
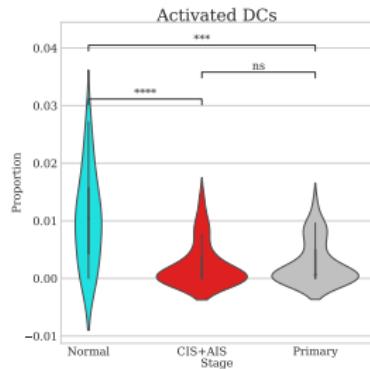
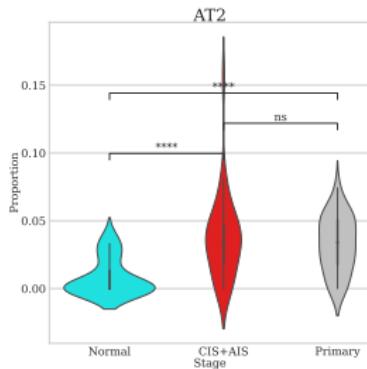


Figure: Cluster Plot in LUSC

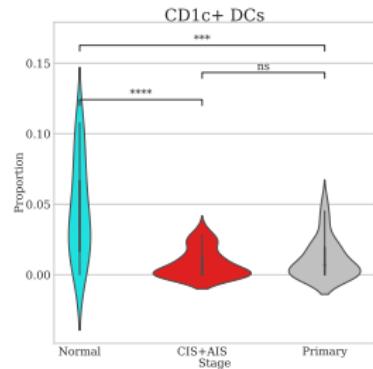
# Violin Plots in LUSC I



(a) Activated DCs



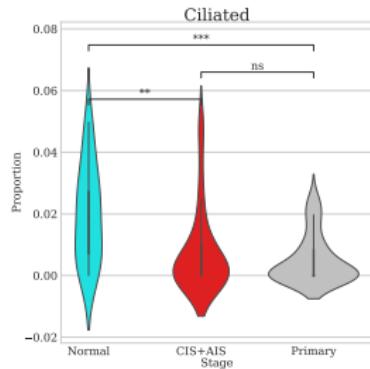
(b) Alveolar type II



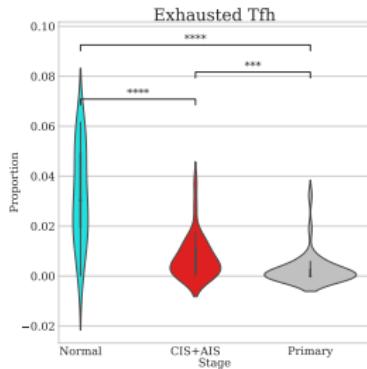
(c) Langerhans cells

Figure: Violin Plots in LUSC

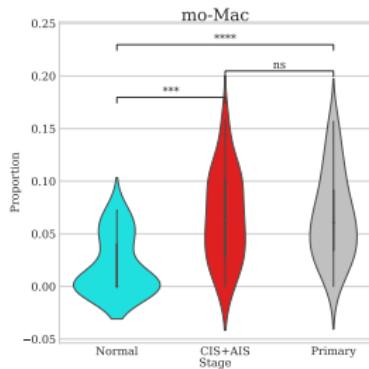
# Violin Plots in LUSC II



(d) Ciliated cells



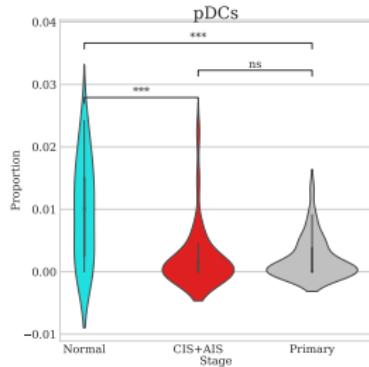
(e) Exhausted T follicular helper



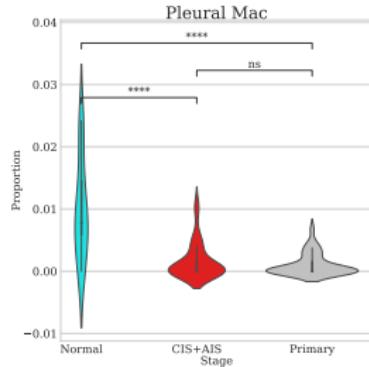
(f) Mo & Mac

Figure: Violin Plots in LUSC

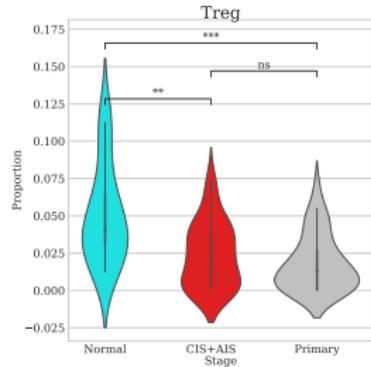
# Violin Plots in LUSC III



(g) Plasmacytoid DCs



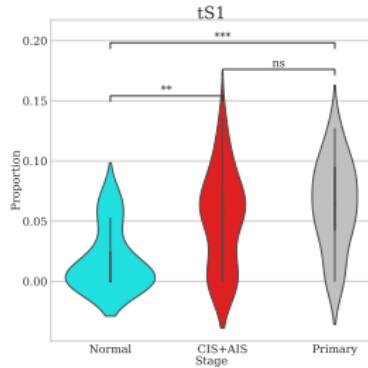
(h) Pleural Mac



(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).

# Findings in Bulk Cell Deconvolution with LUSC IV

## 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

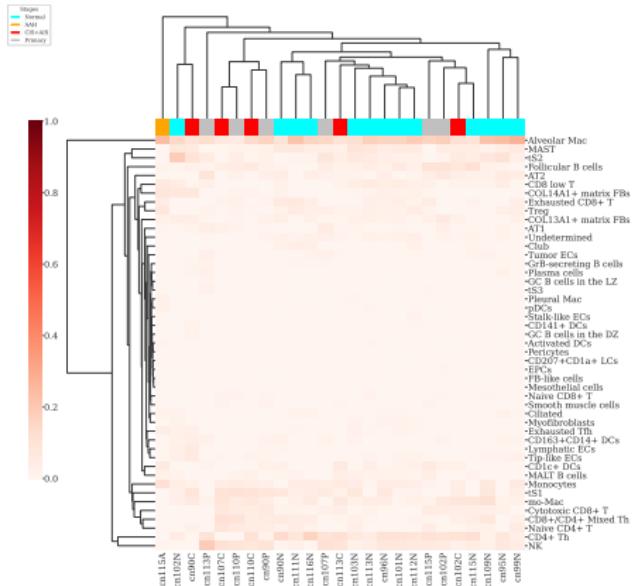
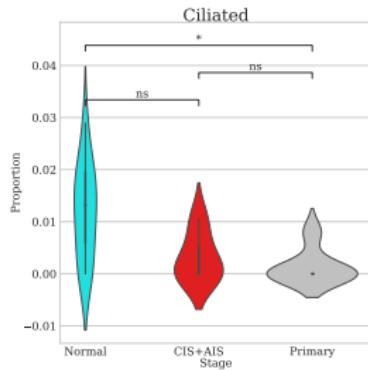
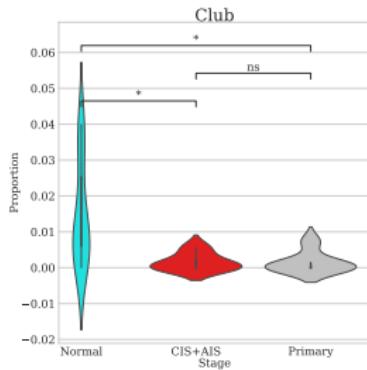


Figure: Cluster Plot in LUAD

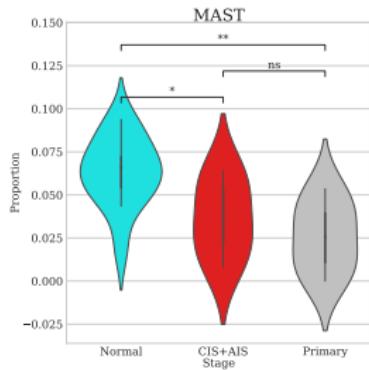
# Violin Plots in LUAD I



(a) Ciliated cells



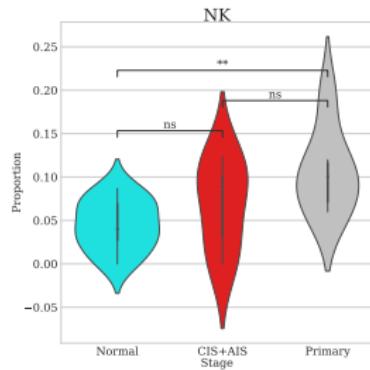
(b) Club Cell



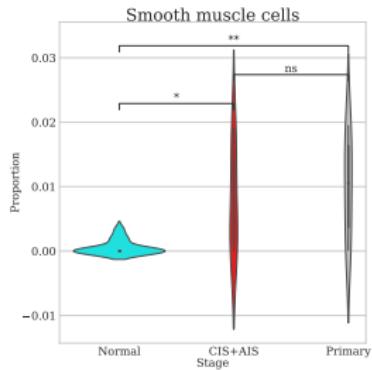
(c) Mast cell

Figure: Violin Plots in LUAD

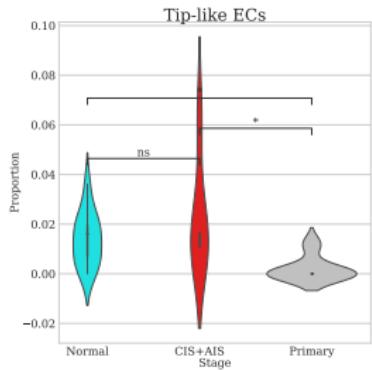
# Violin Plots in LUAD II



(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

### 4.8. Bulk Cell Deconvolution

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

# Reference Single-cell Data

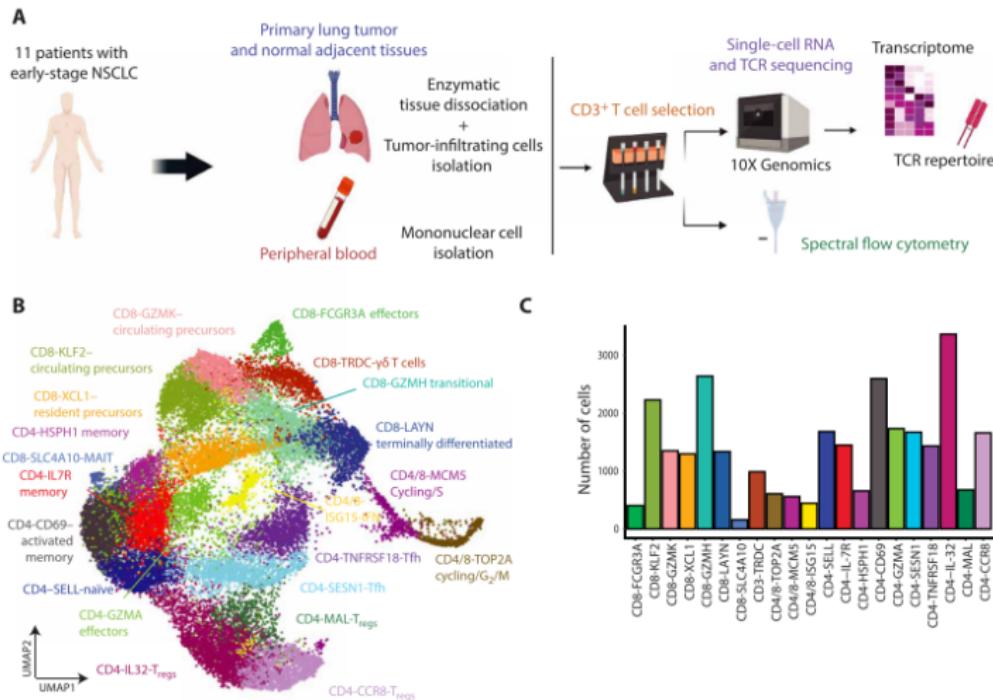


Figure: Characterization of CD3<sup>+</sup> TILs in NSCLC (Gueguen et al., 2021)

# Cluster Plots in LUSC

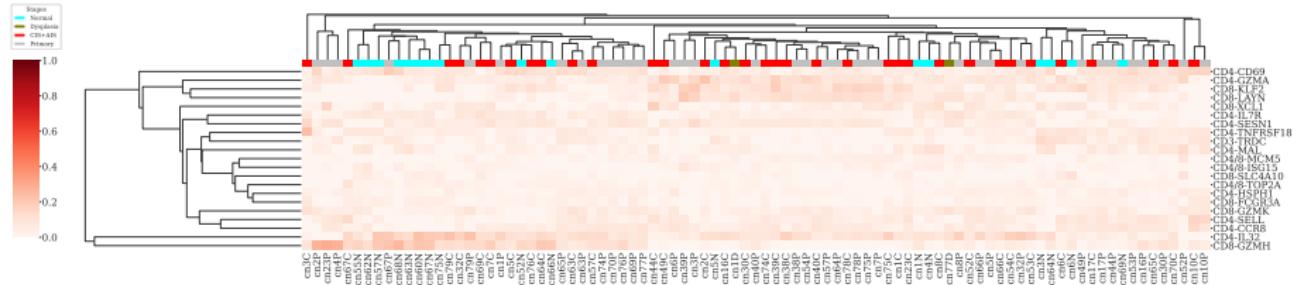
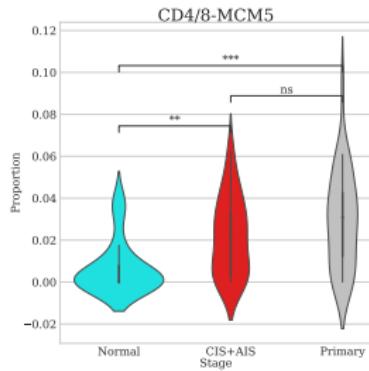
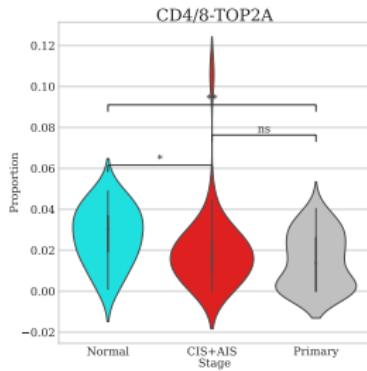


Figure: Cluster Plot in LUAD

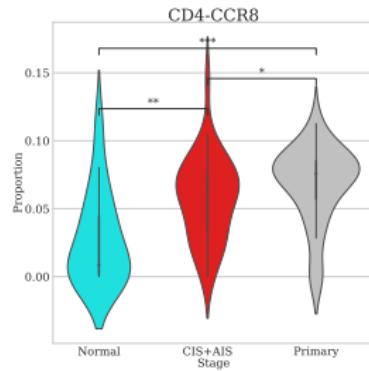
# Violin Plots in LUSC I



(a) CD4/8-MCM5



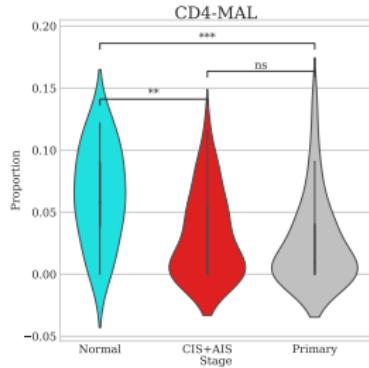
(b) CD4/8-TOP2A



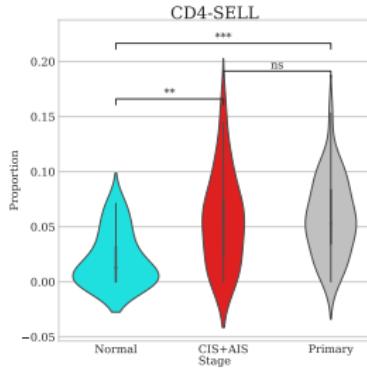
(c) CD4-CCR8

Figure: Violin Plots in LUSC

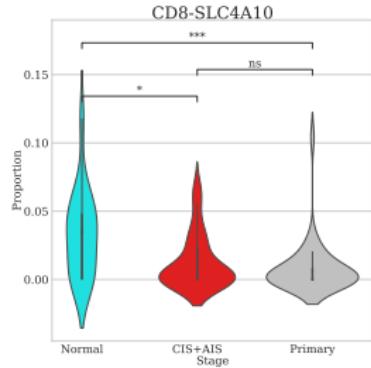
# Violin Plots in LUSC II



(d) CD4-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

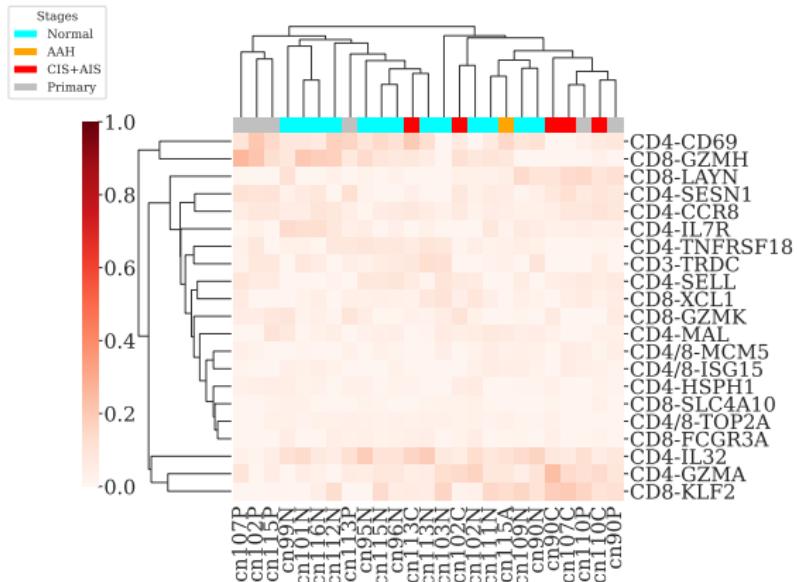
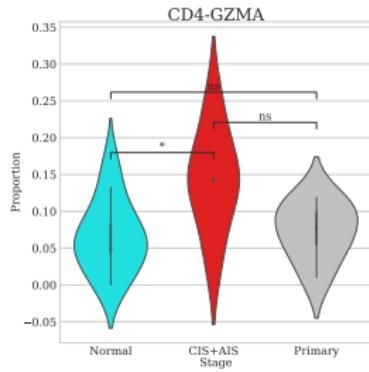
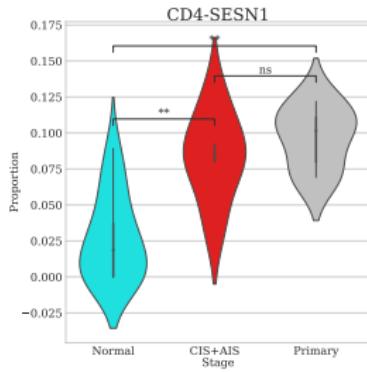


Figure: Cluster Plot in LUAD

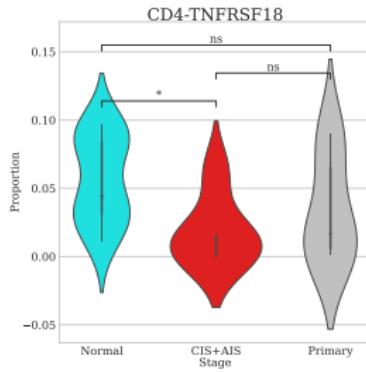
# Violin Plots in LUAD



(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

### 4.9. Discovery of Mutational Signature

# Mutational Signature?

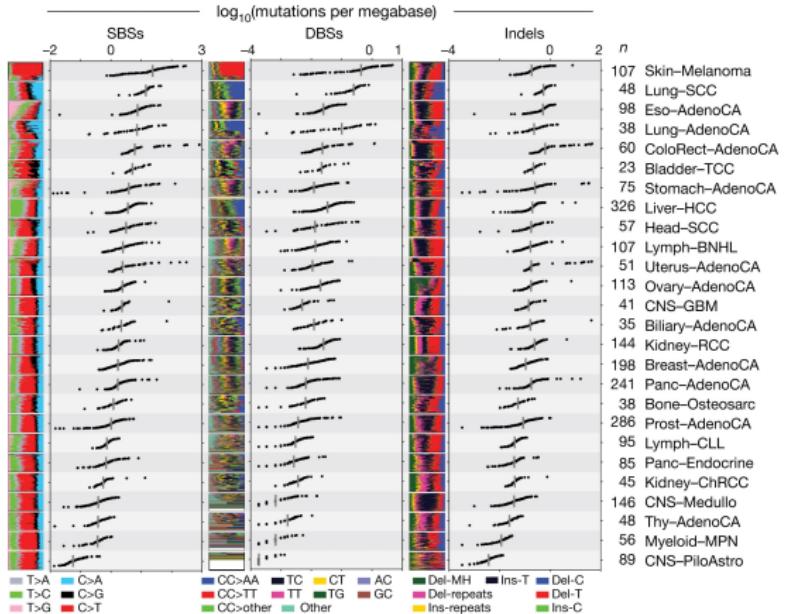
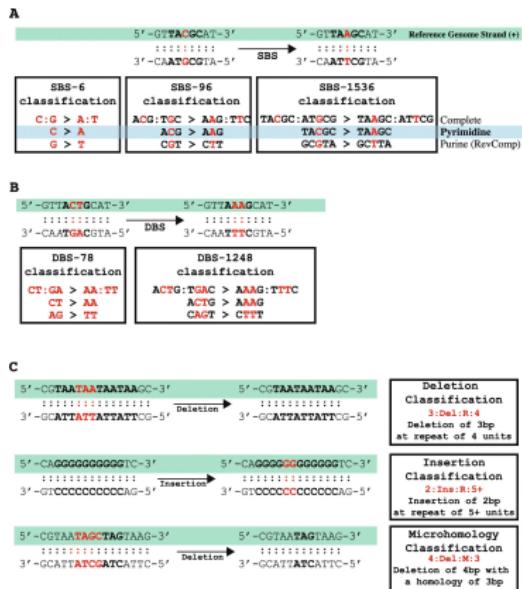


Figure: Mutation Burdens across PCAWG tumor types (Alexandrov et al., 2020)

# SigProfiler?



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

## 4. Results

### 4.9. Discovery of Mutational Signature

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

# SBS Signatures I

## SBS1

- An endogenous mutational process (Nik-Zainal et al., 2012)
- 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., 2012)
  - ① APOBEC3A is probably responsible in human cancer
  - ② APOBEC3B may also contribute
- may be generated directly by DNA replication

# SBS Signatures II

## SBS4

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

## SBS5

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

## SBS10b

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

# SBS Signatures III

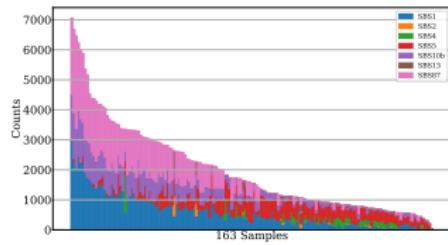
## SBS13

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

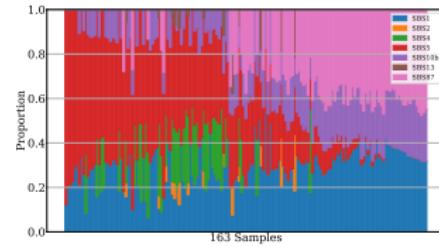
## SBS87

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

# SBS in LUSC I



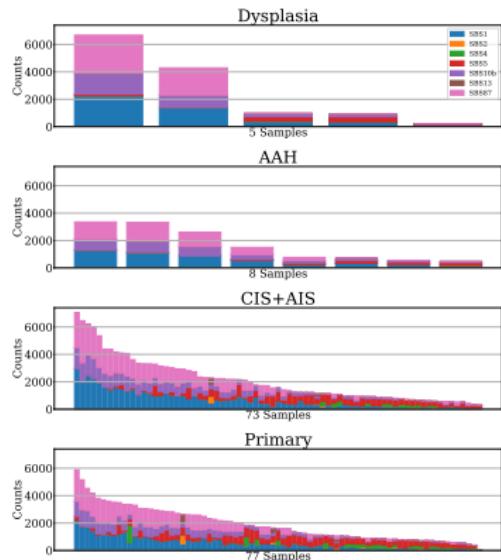
(a) Absolute



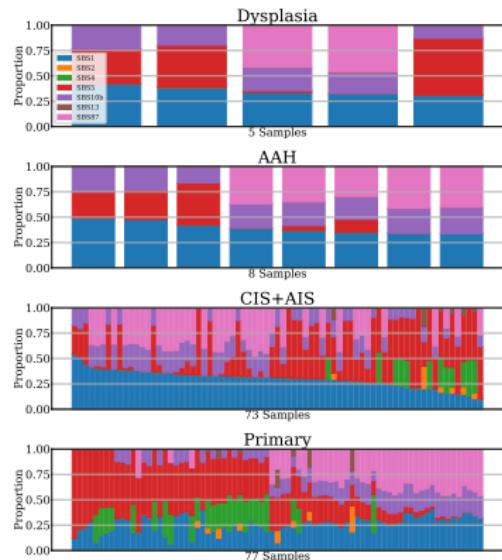
(b) Relative

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

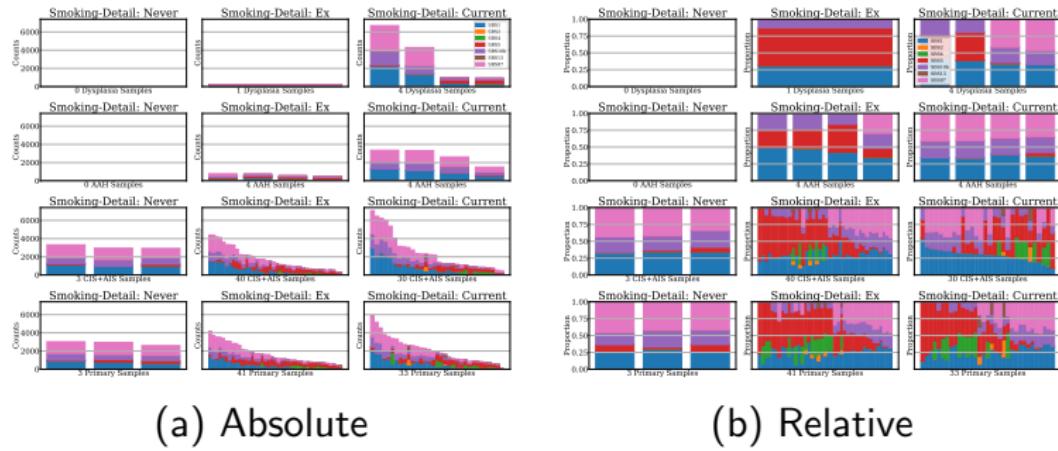
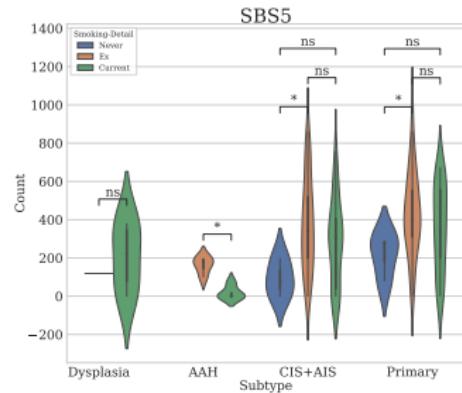
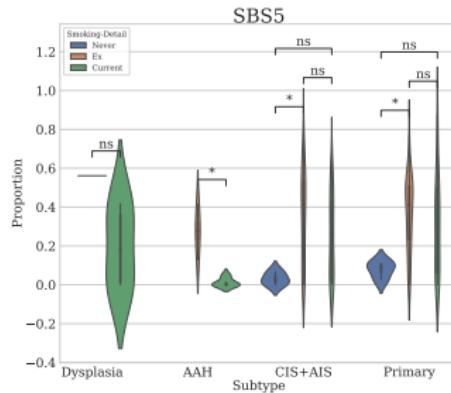


Figure: SBS Bar Plot by Cancer Subtype & Smoking in LUSC

# SBS in LUSC with Smoking II



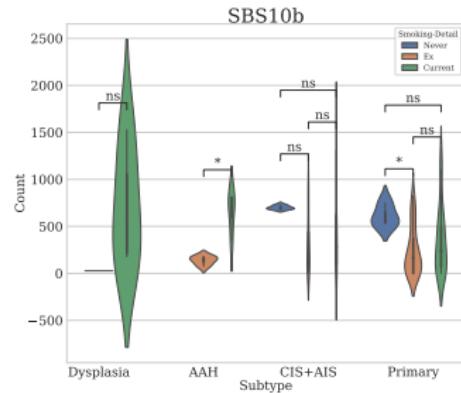
(a) Absolute



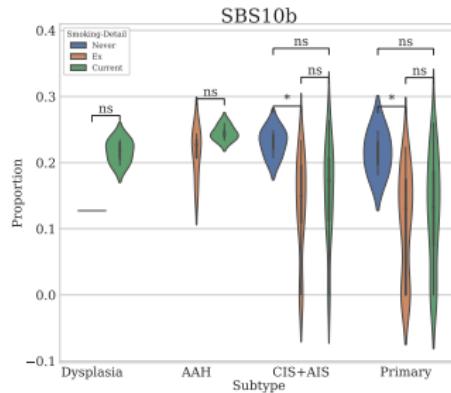
(b) Relative

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

# SBS in LUSC with Smoking III



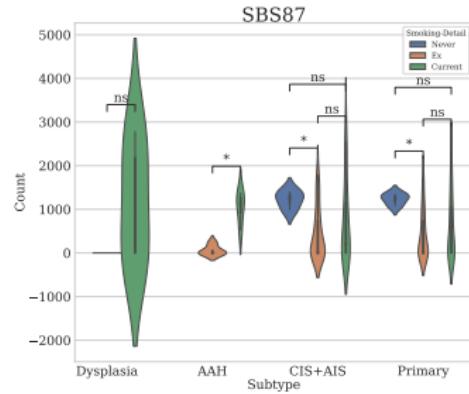
(a) Absolute



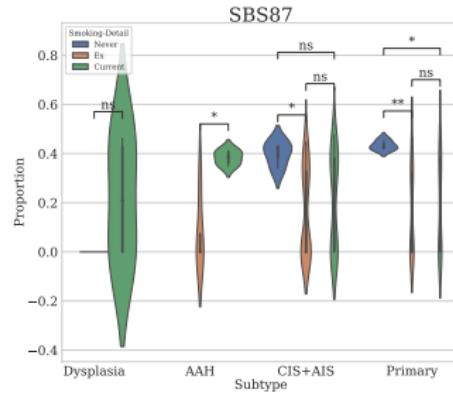
(b) Relative

Figure: SBS10b Signature in LUSC with Smoking

# SBS in LUSC with Smoking IV



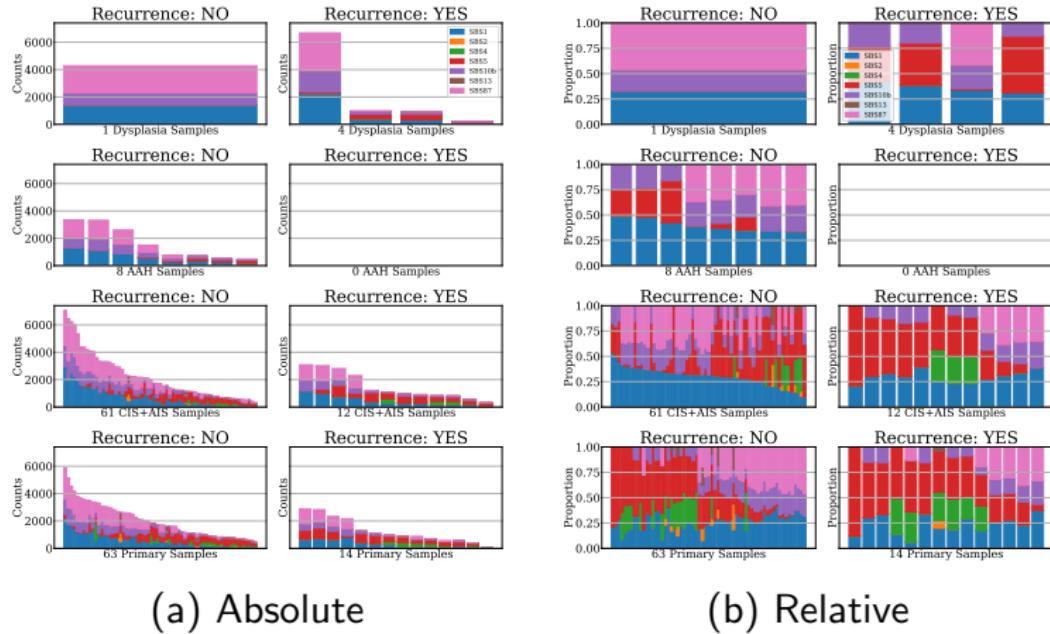
(a) Absolute



(b) Relative

Figure: SBS87 Signature in LUSC with Smoking

# SBS in LUSC with Recurrence I

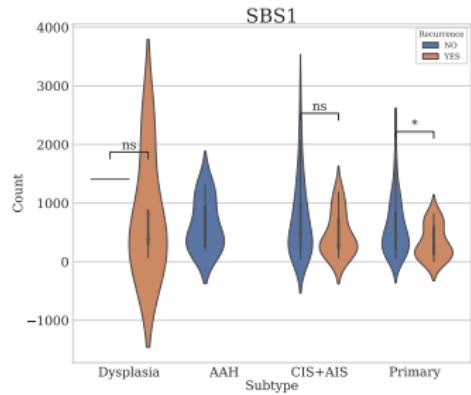


(a) Absolute

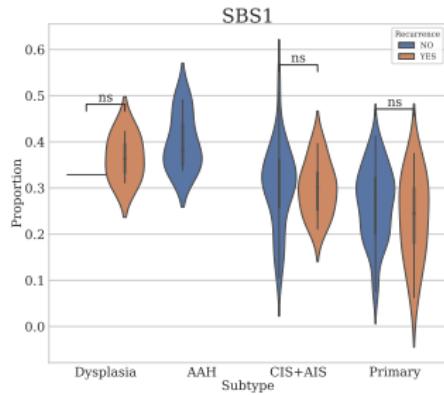
(b) Relative

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

# SBS in LUSC with Recurrence II



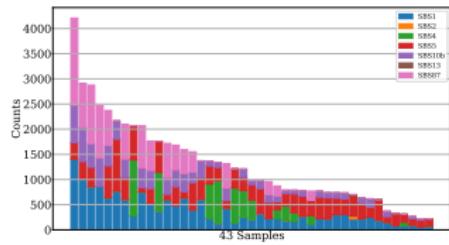
(a) Absolute



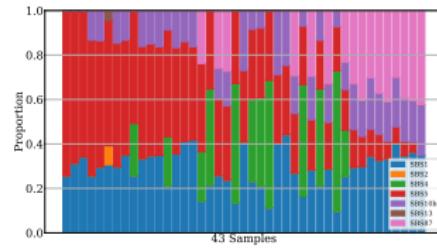
(b) Relative

Figure: SBS1 Signature in LUSC with Recurrence

# SBS in LUAD I



(a) Absolute



(b) Relative

Figure: SBS Bar Plot in LUSC

# SBS in LUAD II

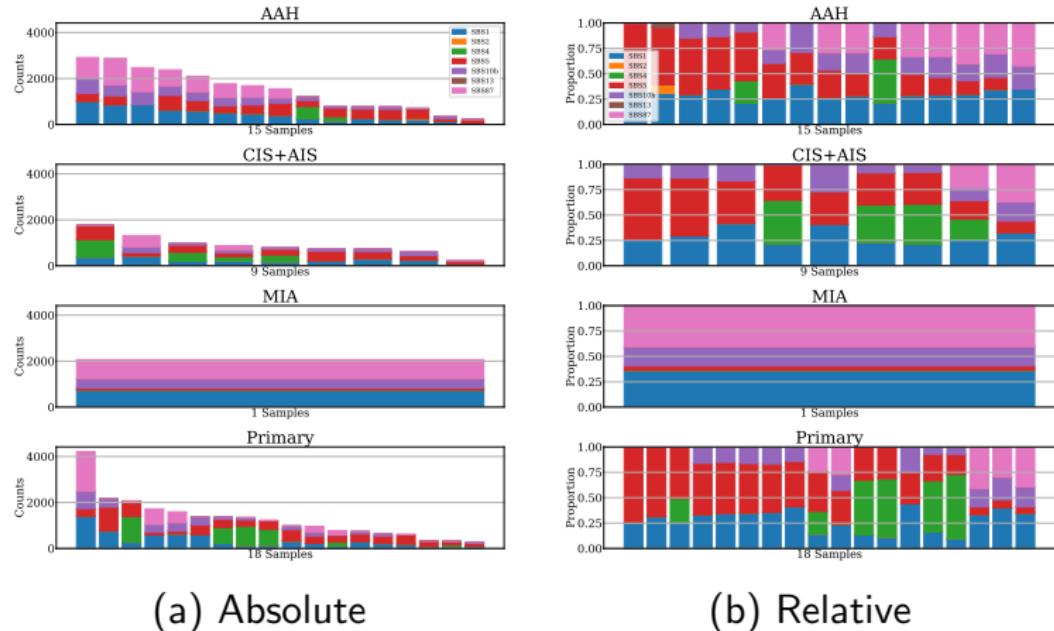


Figure: SBS Bar Plot by Cancer Subtype in LUSC

# SBS in LUAD with Smoking I

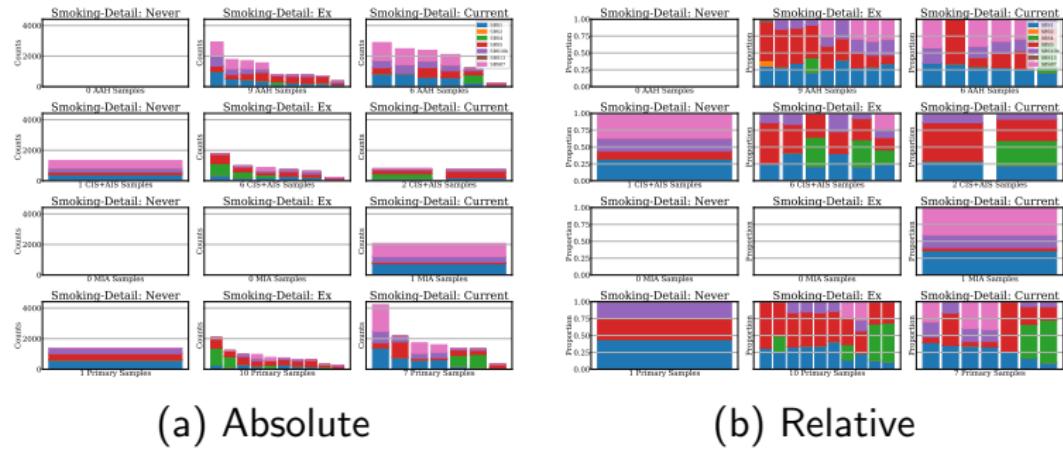
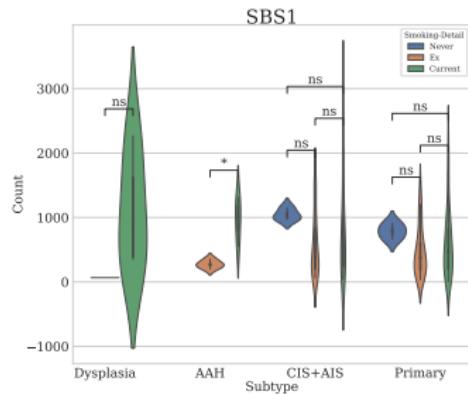
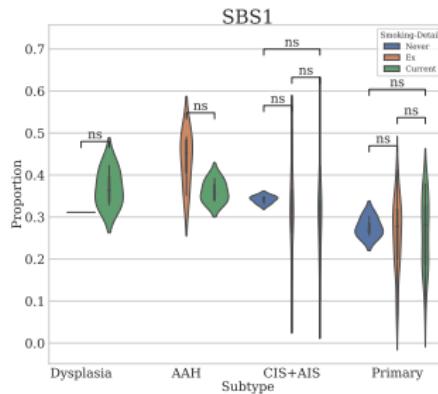


Figure: SBS Bar Plot by Cancer Subtype & Smoking in LUAD

# SBS in LUAD with Smoking II



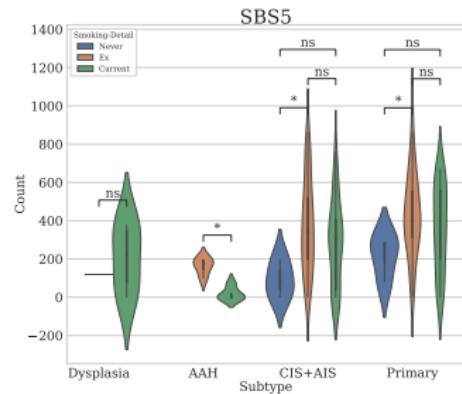
(a) Absolute



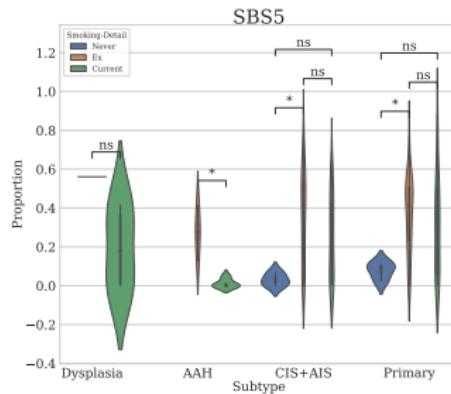
(b) Relative

Figure: SBS1 Signature in LUAD with Smoking

# SBS in LUAD with Smoking III



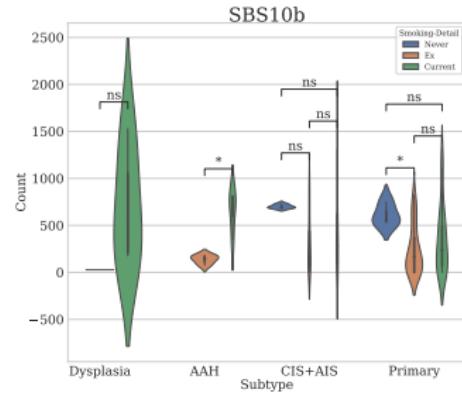
(a) Absolute



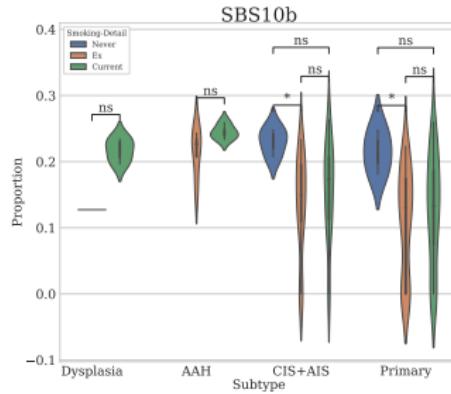
(b) Relative

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

# SBS in LUAD with Smoking IV



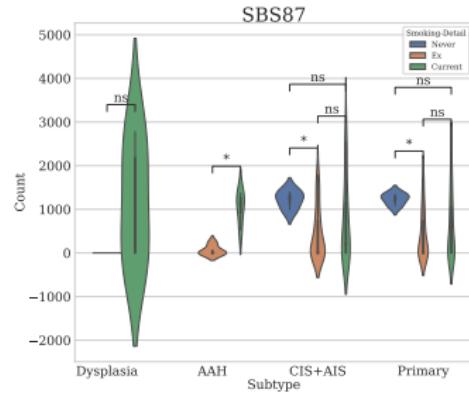
(a) Absolute



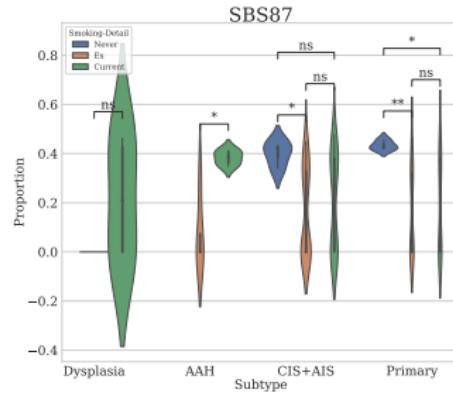
(b) Relative

Figure: SBS10b Signature in LUAD with Smoking

# SBS in LUAD with Smoking V



(a) Absolute



(b) Relative

Figure: SBS87 Signature in LUAD with Smoking

# SBS in LUAD with Recurrence I

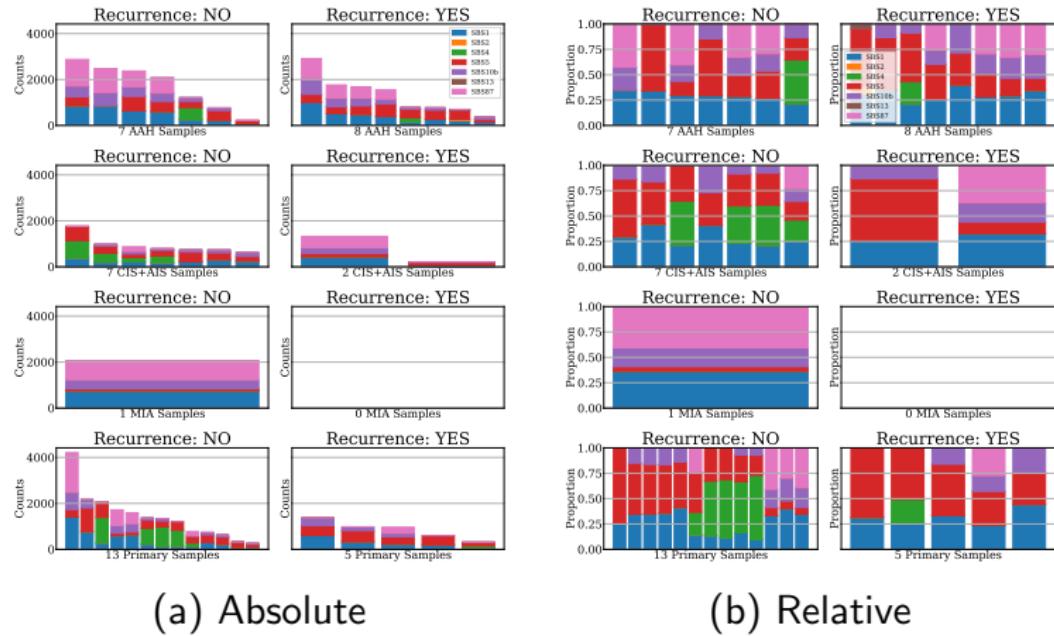
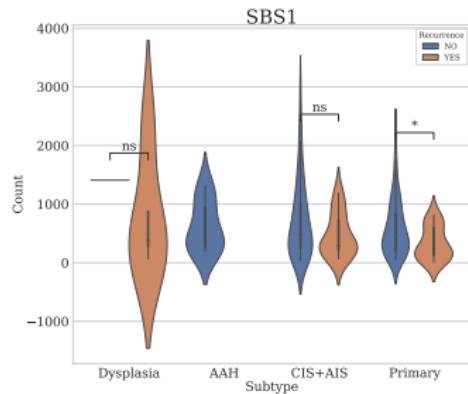
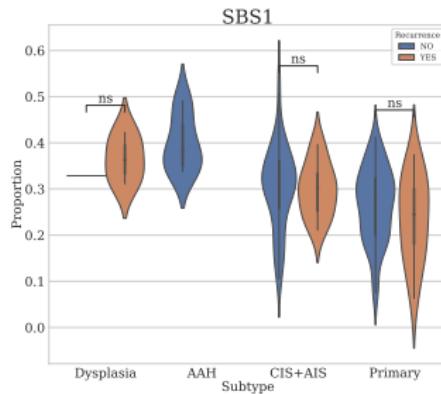


Figure: SBS Bar Plot by Cancer Subtype & Recurrence in LUAD

# SBS in LUAD with Recurrence II



(a) Absolute



(b) Relative

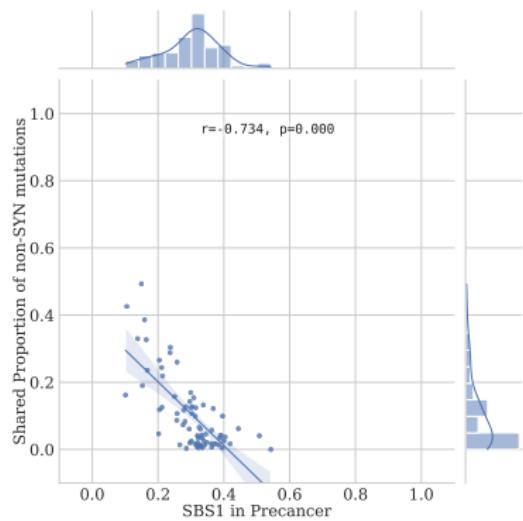
Figure: SBS1 Signature in LUAD with Recurrence

## 4. Results

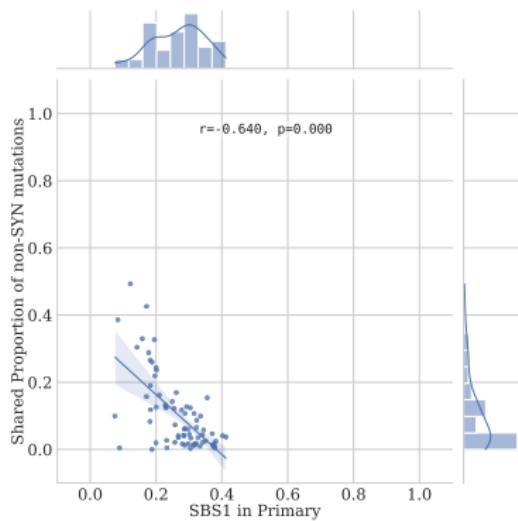
### 4.9. Discovery of Mutational Signature

#### 4.9.2. SBS with Shared Mutation Proportion in LUSC

# SBS1 with Shared Mutation Proportion in LUSC I



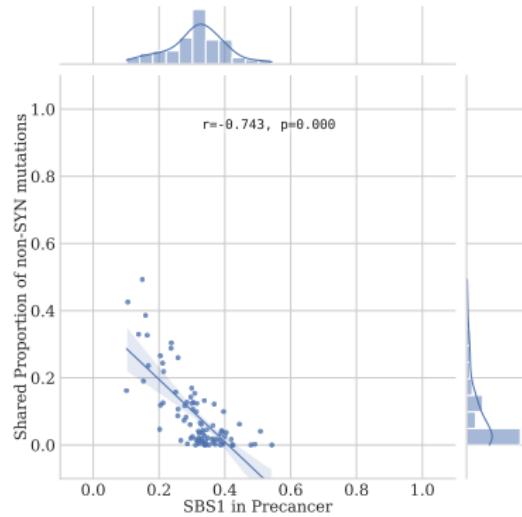
(a) Precancer score



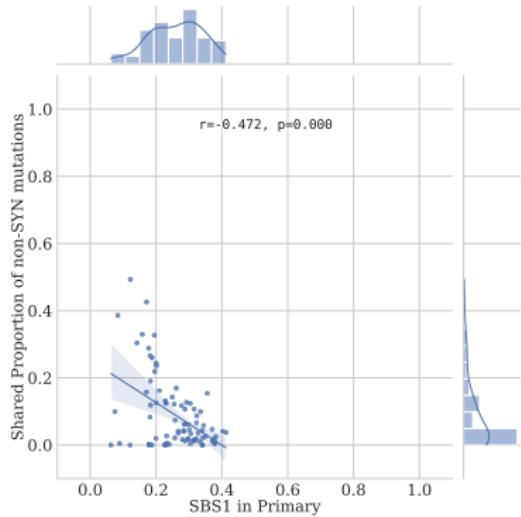
(b) Primary score

Figure: SBS1 with CIS samples

# SBS1 with Shared Mutation Proportion in LUSC II



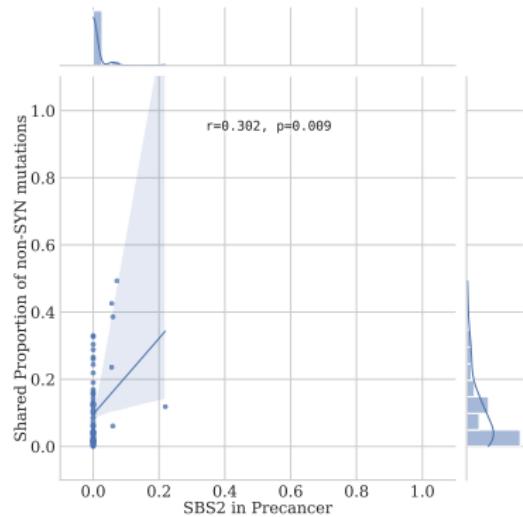
(a) Precancer score



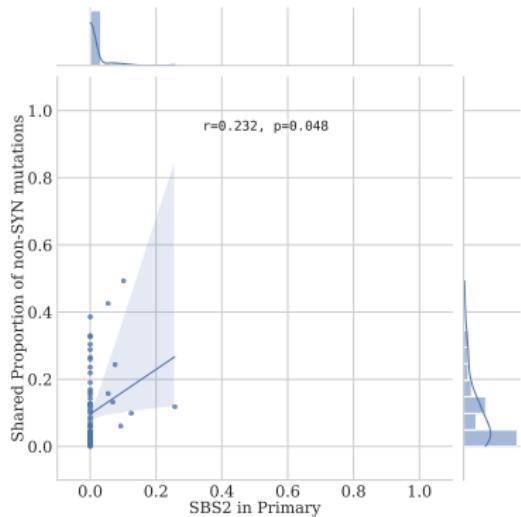
(b) Primary score

Figure: SBS1 with Precancer samples

# SBS2 with Shared Mutation Proportion in LUSC I



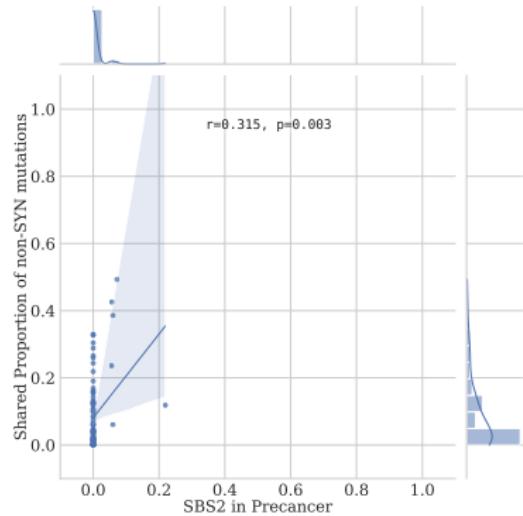
(a) Precancer score



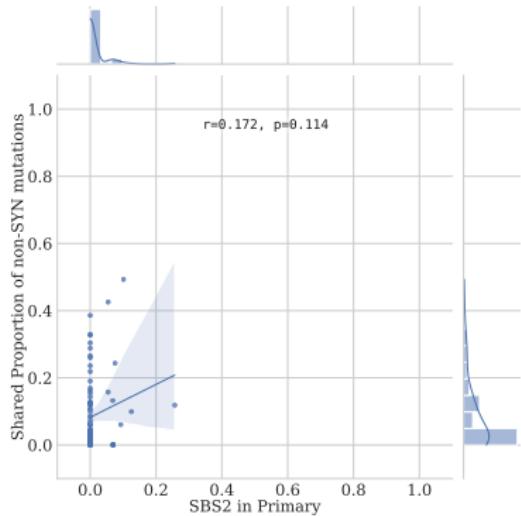
(b) Primary score

Figure: SBS2 with CIS samples

# SBS2 with Shared Mutation Proportion in LUSC II



(a) Precancer score



(b) Primary score

Figure: SBS2 with Precancer samples

# SBS4 with Shared Mutation Proportion in LUSC I

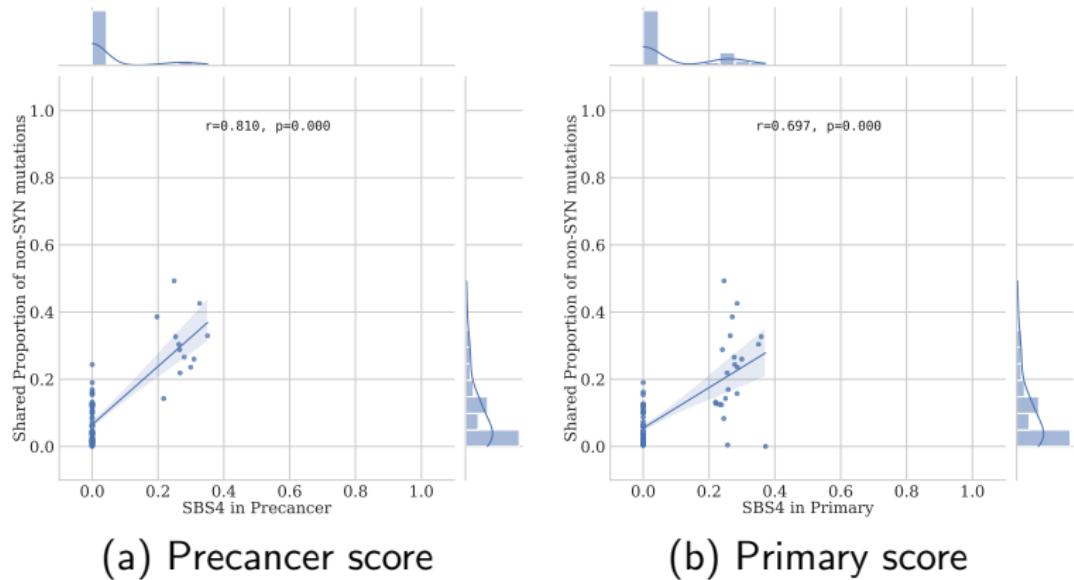
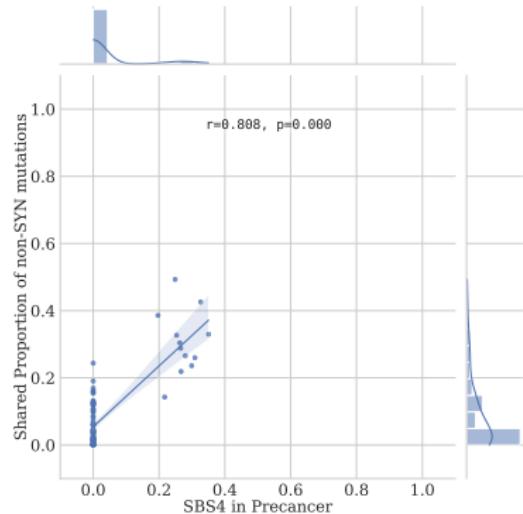
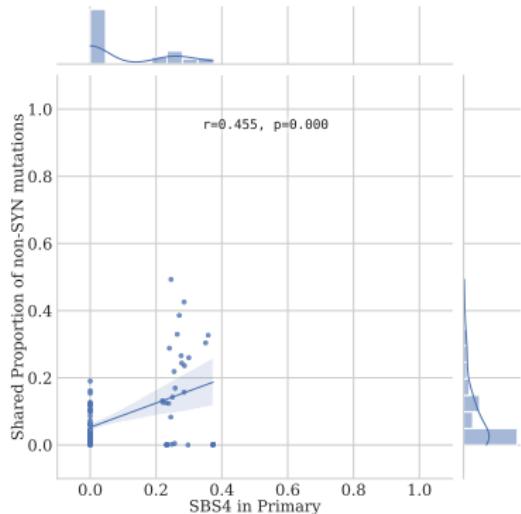


Figure: SBS4 with CIS samples

# SBS4 with Shared Mutation Proportion in LUSC II



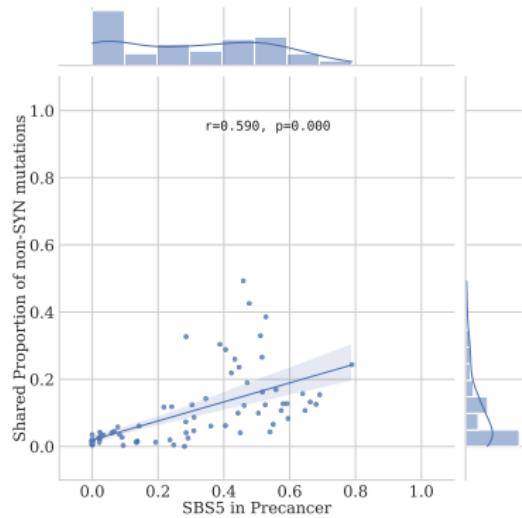
(a) Precancer score



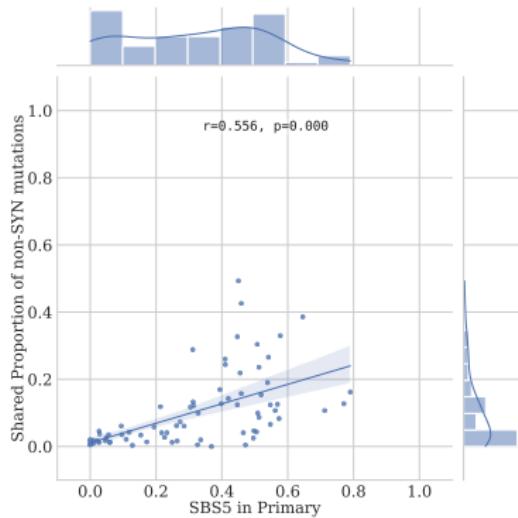
(b) Primary score

Figure: SBS4 with Precancer samples

# SBS5 with Shared Mutation Proportion in LUSC I



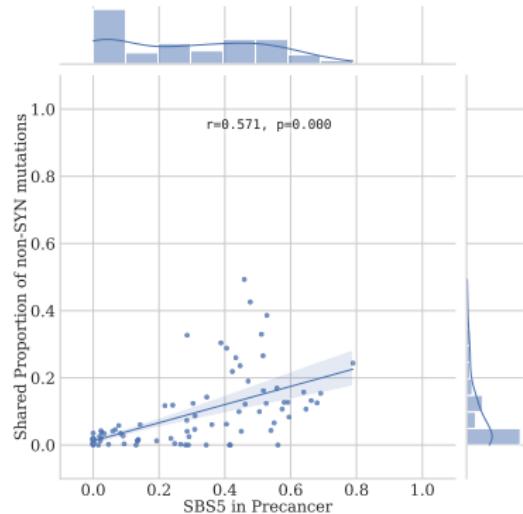
(a) Precancer score



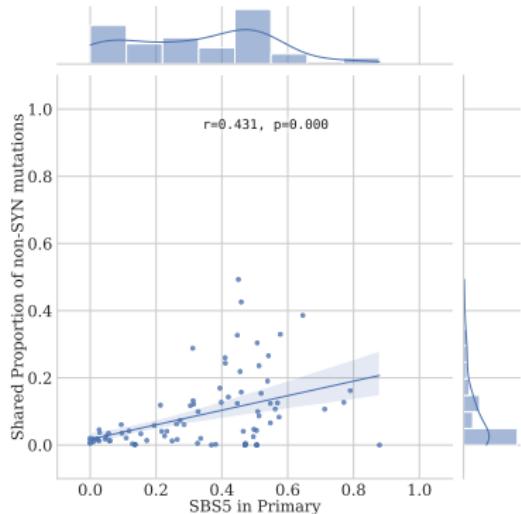
(b) Primary score

Figure: SBS5 with CIS samples

# SBS5 with Shared Mutation Proportion in LUSC II



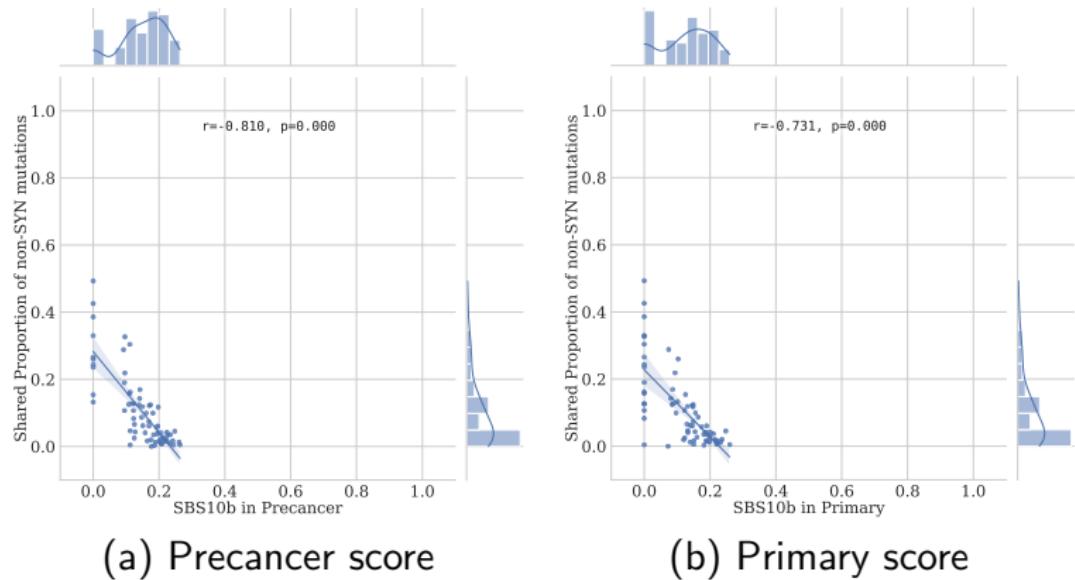
(a) Precancer score



(b) Primary score

Figure: SBS5 with Precancer samples

# SBS10b with Shared Mutation Proportion in LUSC I



**Figure:** SBS10b with CIS samples

# SBS10b with Shared Mutation Proportion in LUSC II

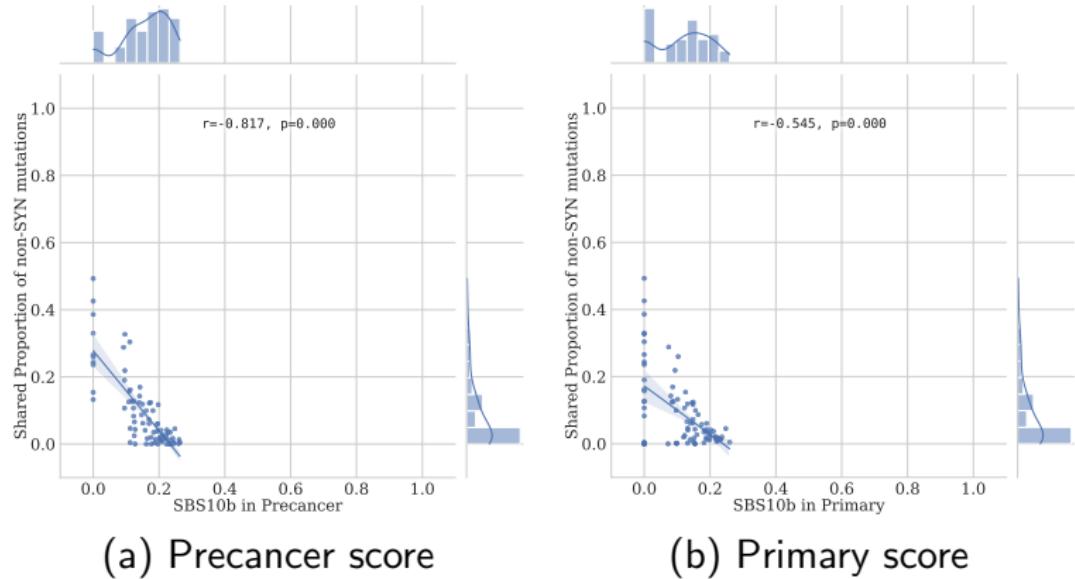
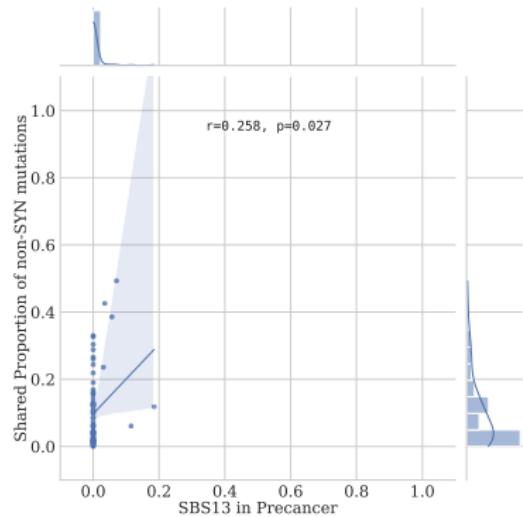
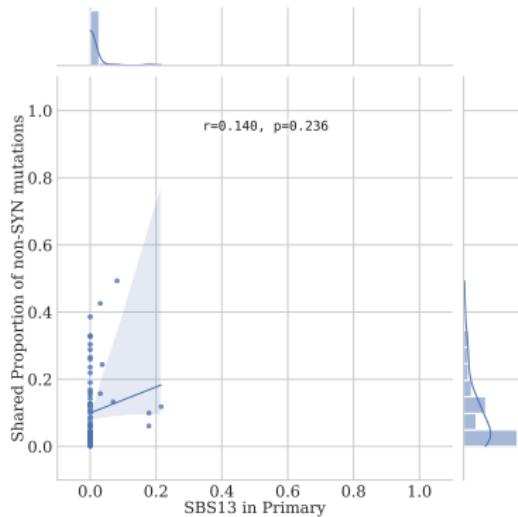


Figure: SBS10b with Precancer samples

# SBS13 with Shared Mutation Proportion in LUSC I



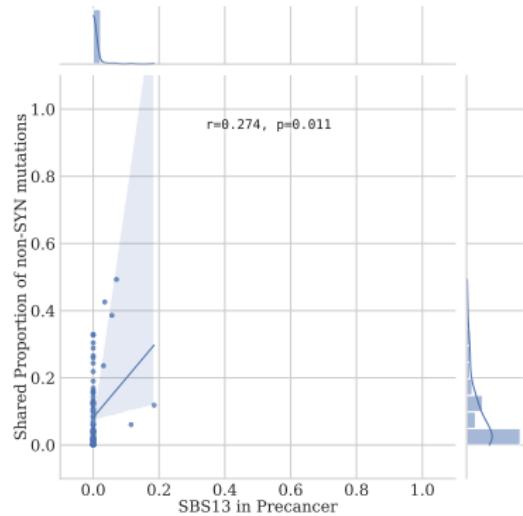
(a) Precancer score



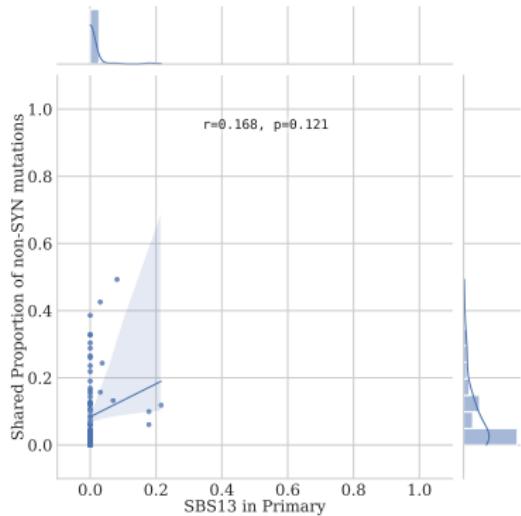
(b) Primary score

Figure: SBS13 with CIS samples

# SBS13 with Shared Mutation Proportion in LUSC II



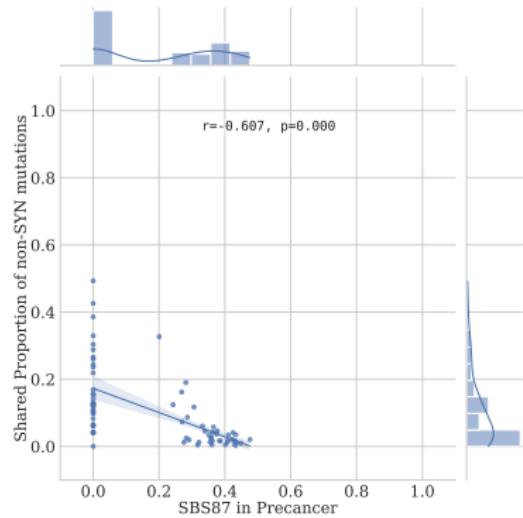
(a) Precancer score



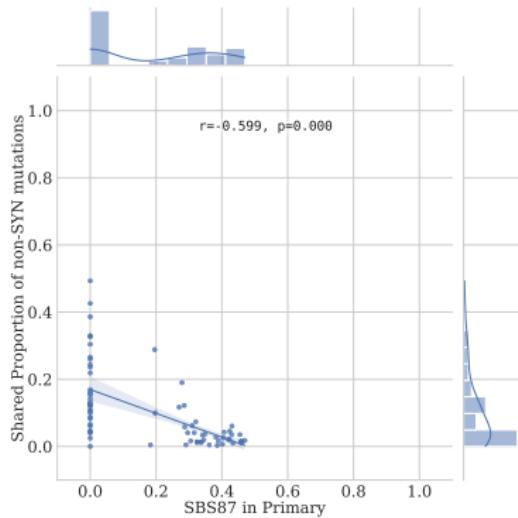
(b) Primary score

Figure: SBS13 with Precancer samples

# SBS87 with Shared Mutation Proportion in LUSC I



(a) Precancer score



(b) Primary score

Figure: SBS87 with CIS samples

# SBS87 with Shared Mutation Proportion in LUSC II

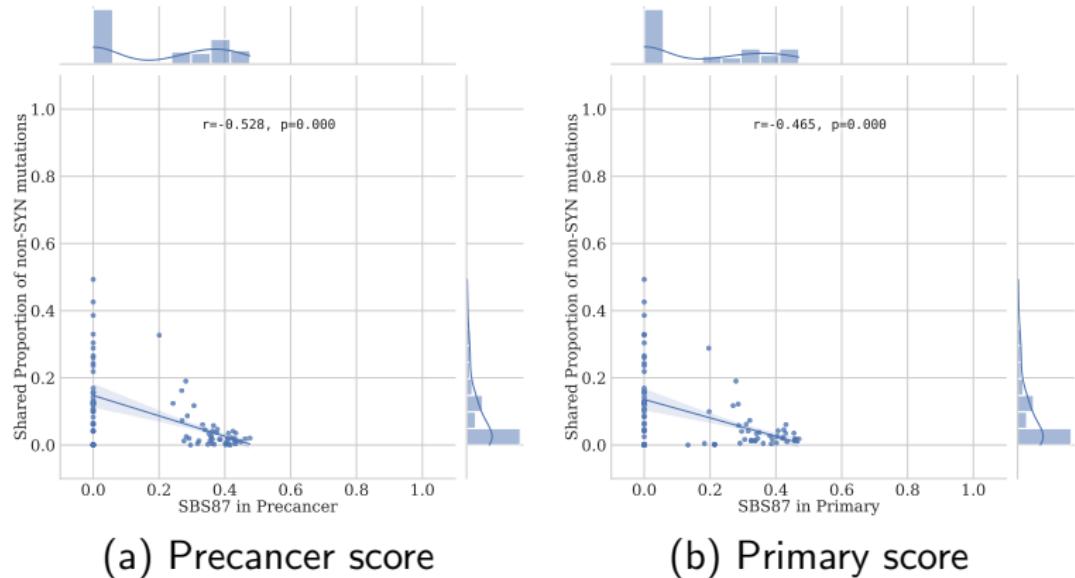


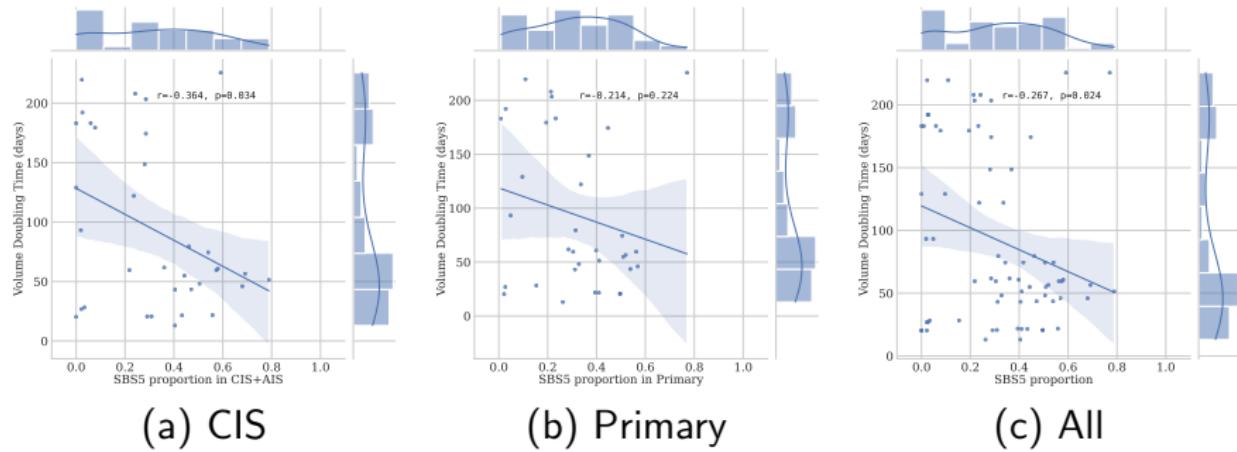
Figure: SBS87 with Precancer samples

## 4. Results

### 4.9. Discovery of Mutational Signature

#### 4.9.3. SBS with Clinical Values in LUSC

# SBS5 with Volume Doubling Time in LUSC



**Figure:** SBS5 with VDT in LUSC

# SBS1 with Recurrence-Free Survival in LUSC

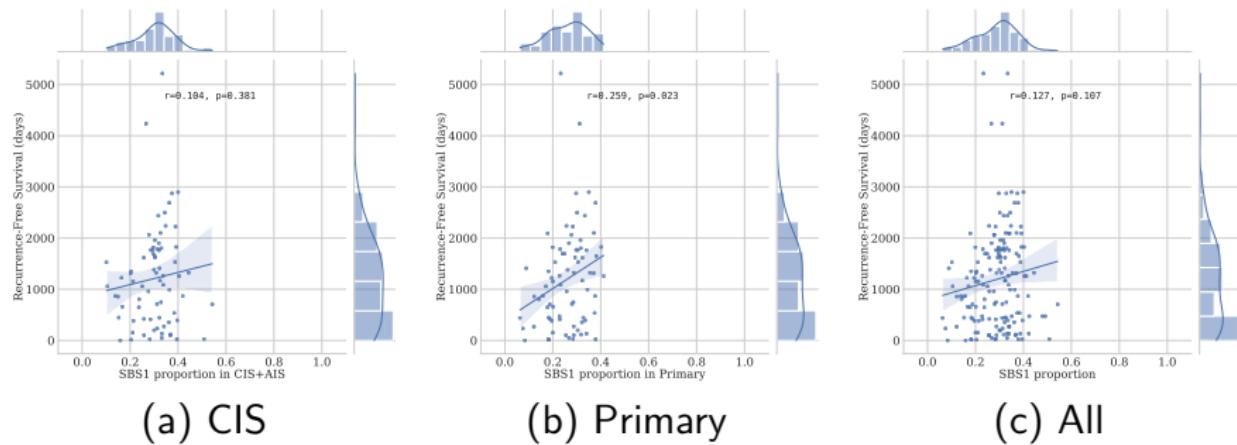


Figure: SBS1 with RFS in LUSC

# SBS5 with Recurrence-Free Survival in LUSC

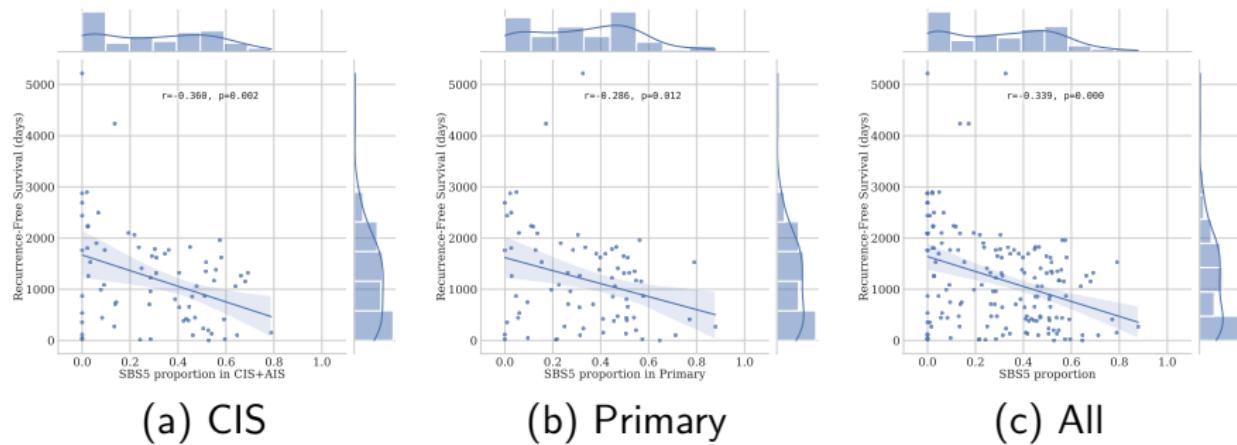
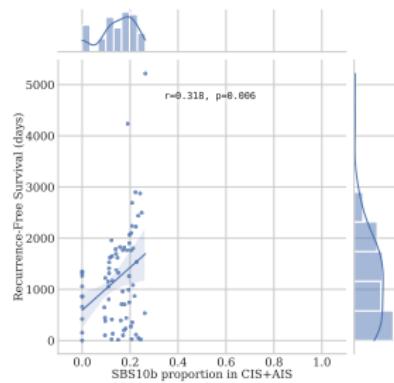
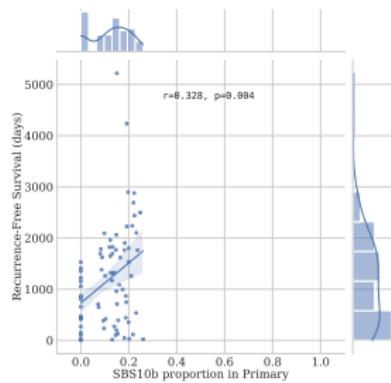


Figure: SBS5 with RFS in LUSC

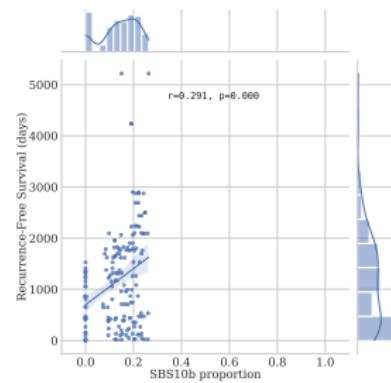
# SBS10b with Recurrence-Free Survival in LUSC



(a) CIS



(b) Primary



(c) All

Figure: SBS10b with RFS in LUSC

# SBS87 with Recurrence-Free Survival in LUSC

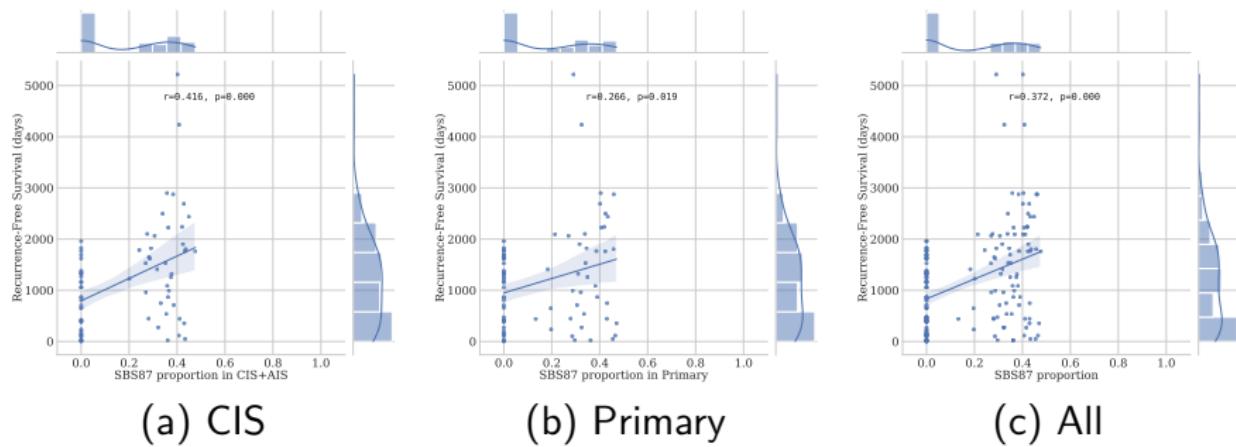


Figure: SBS87 with RFS in LUSC

# SBS1 with Overall Survival in LUSC

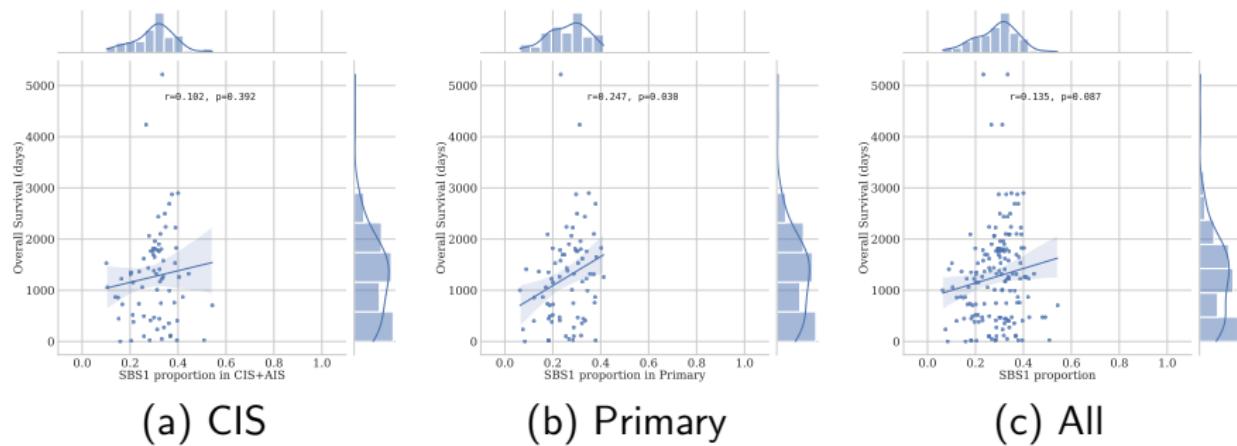


Figure: SBS1 with OS in LUSC

# SBS5 with Overall Survival in LUSC

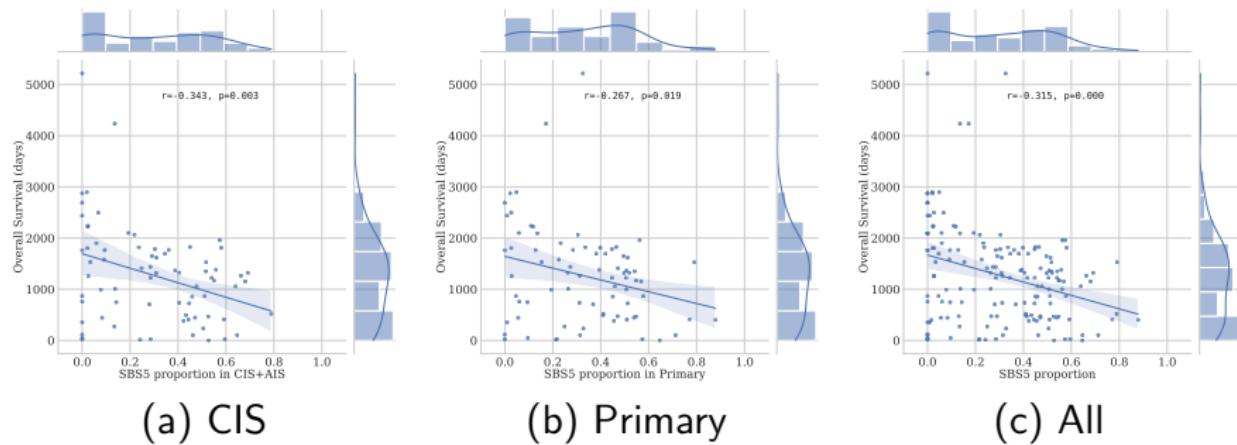


Figure: SBS5 with OS in LUSC

# SBS10b with Overall Survival in LUSC

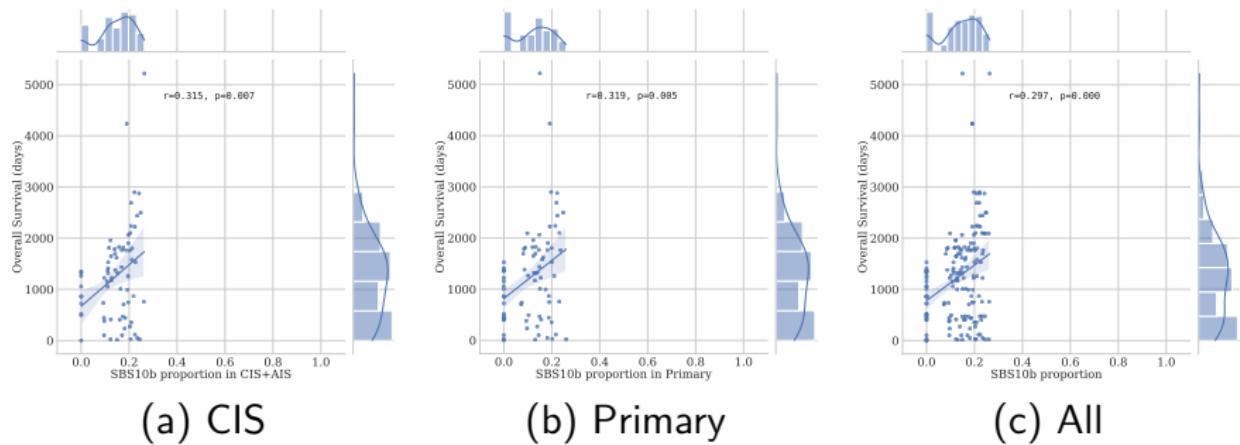


Figure: SBS10b with CIS samples

# SBS87 with Overall Survival in LUSC

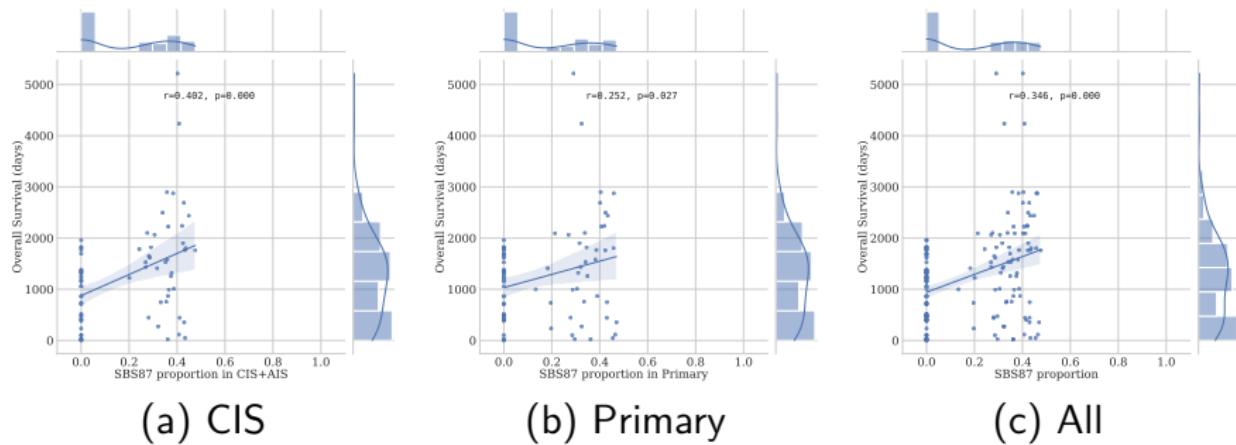


Figure: SBS87 with OS in LUSC

## 4. Results

### 4.9. Discovery of Mutational Signature

#### 4.9.4. SBS with Clinical Values in LUAD

# SBS5 with Overall Survival in LUAD

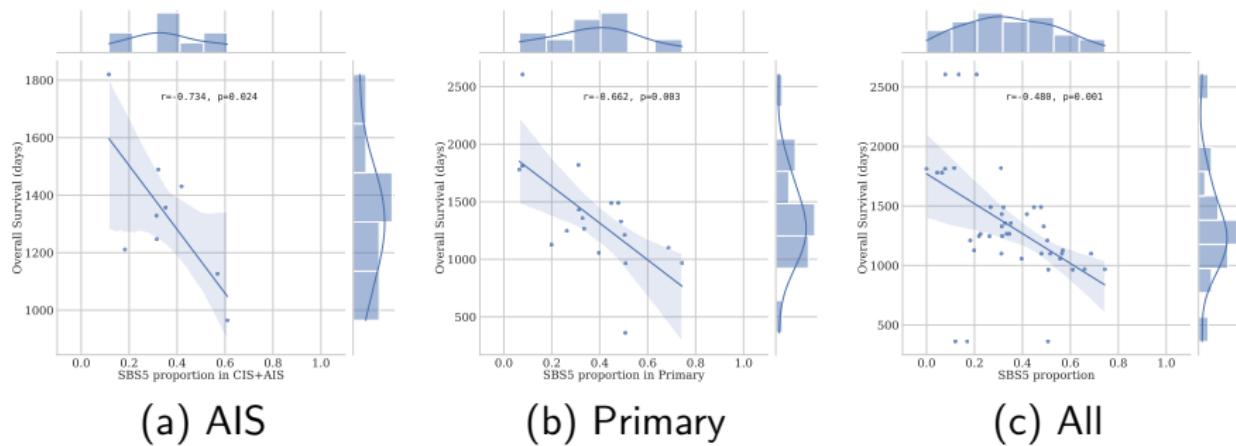


Figure: SBS5 with OS in LUAD

## 4. Results

### 4.9. Discovery of Mutational Signature

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

# DBS Signatures I

## DBS2

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

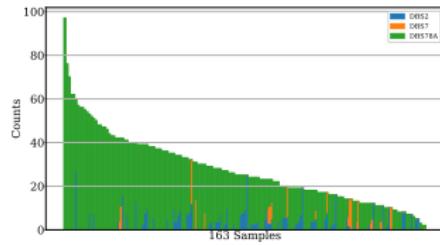
## DBS7

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

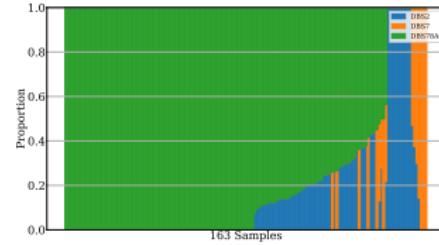
## DBS78A

- Unknown

# DBS in LUSC I



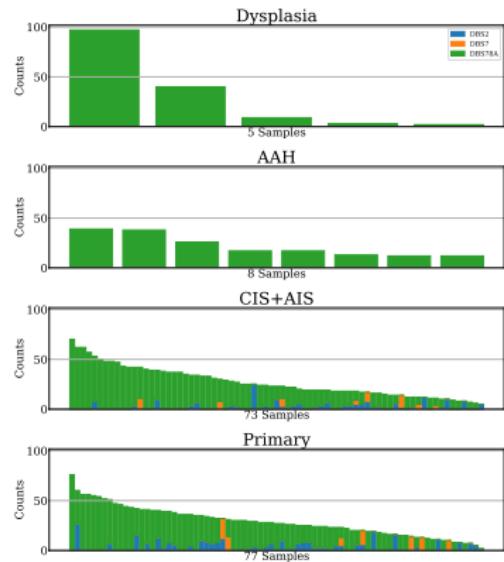
(a) Absolute



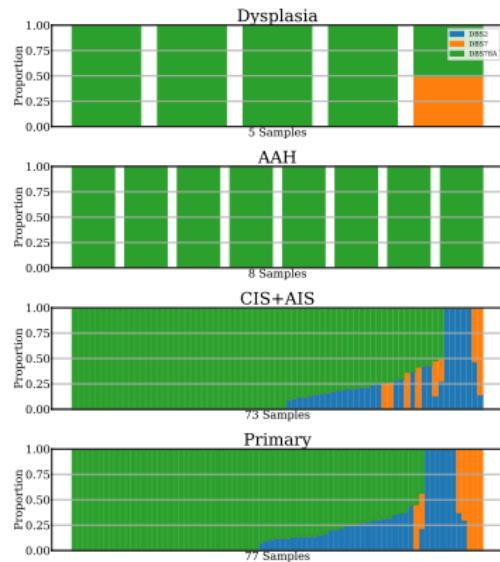
(b) Relative

Figure: DBS Bar Plot in LUSC

# DBS in LUSC II



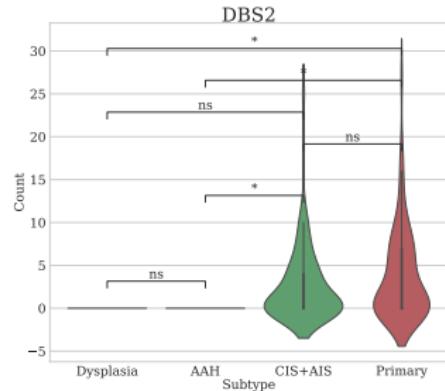
(a) Absolute



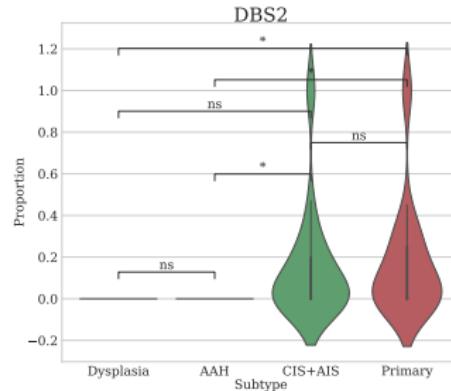
(b) Relative

Figure: DBS Bar Plot by Cancer Subtype in LUSC

# DBS in LUSC III



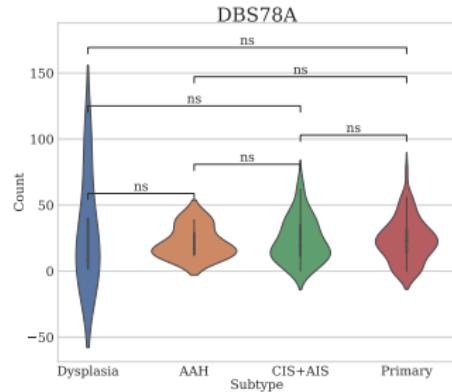
(a) Absolute



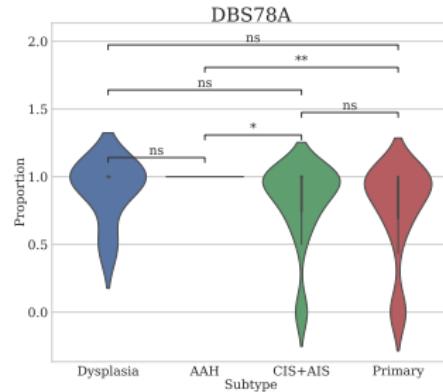
(b) Relative

Figure: DBS2 Signature in LUSC

# DBS in LUSC IV



(a) Absolute



(b) Relative

Figure: DBS78A Signature in LUSC

# DBS in LUSC with Smoking I

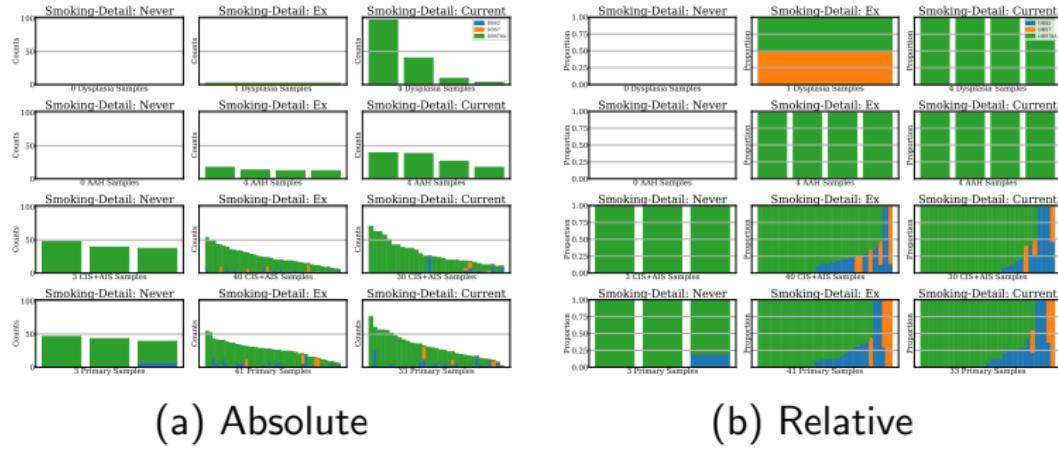
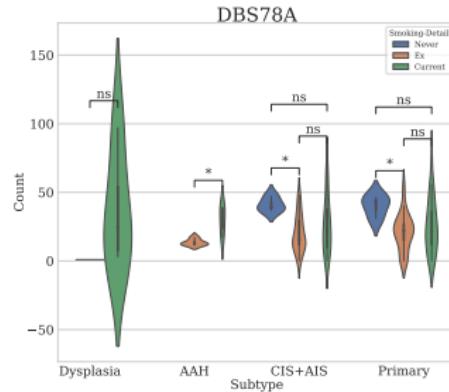
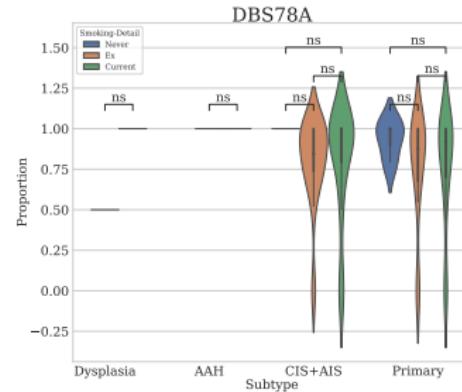


Figure: DBS Bar Plot by Cancer Subtype & Smoking in LUSC

# DBS in LUSC with Smoking II



(a) Absolute



(b) Relative

Figure: DBS78A Signature in LUSC with Smoking

# DBS in LUSC with Recurrence

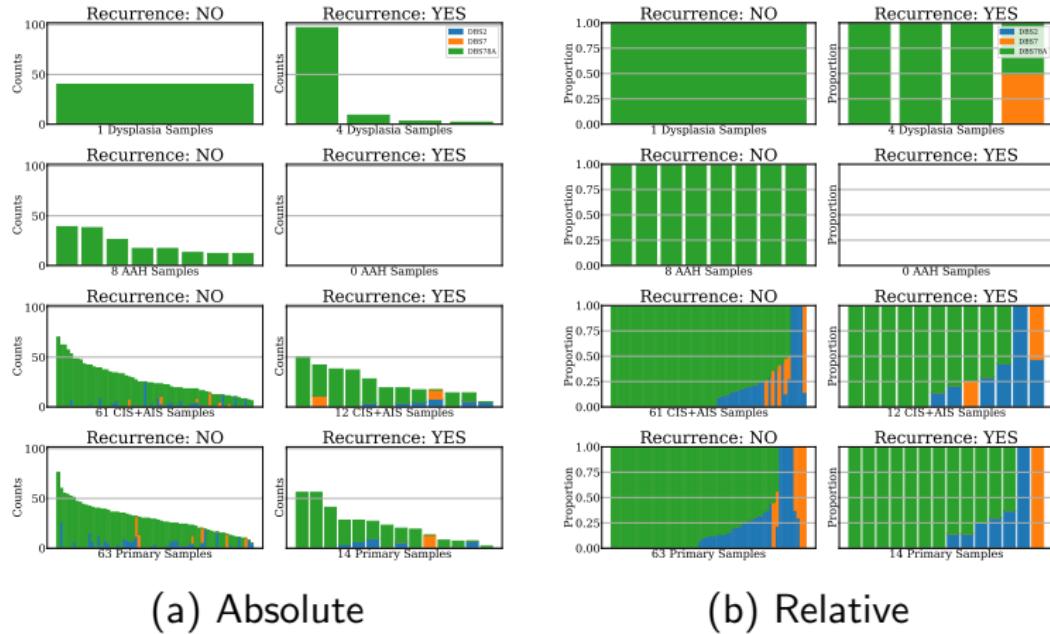
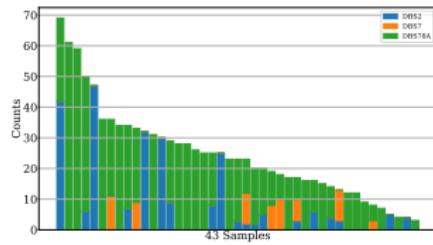
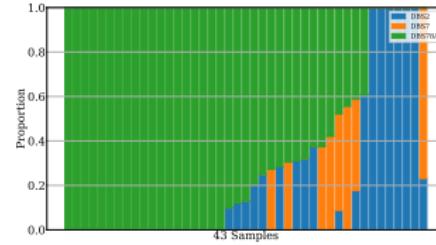


Figure: DBS Bar Plot by Cancer Subtype & Recurrence in LUSC

# DBS in LUAD I



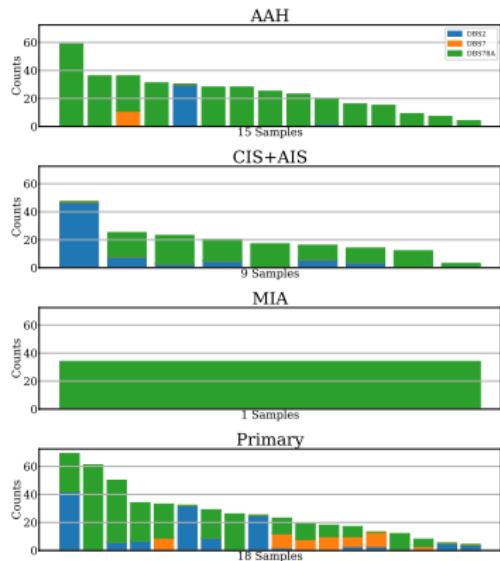
(a) Absolute



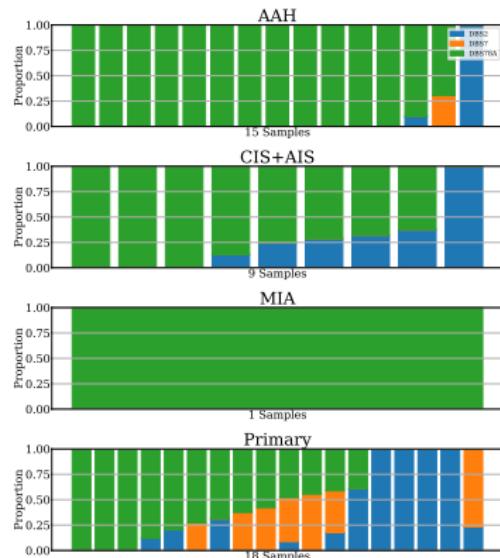
(b) Relative

Figure: DBS Bar Plot in LUAD

# DBS in LUAD II



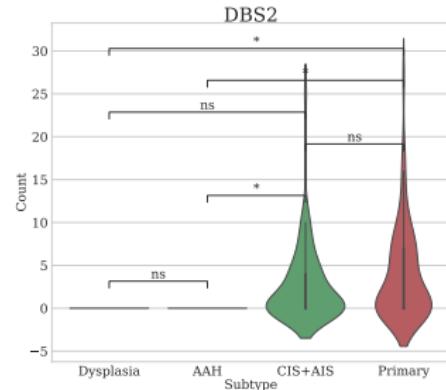
(a) Absolute



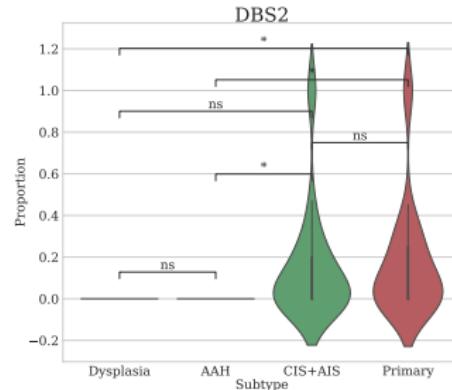
(b) Relative

Figure: DBS Bar Plot by Cancer Subtype in LUAD

# DBS in LUAD III



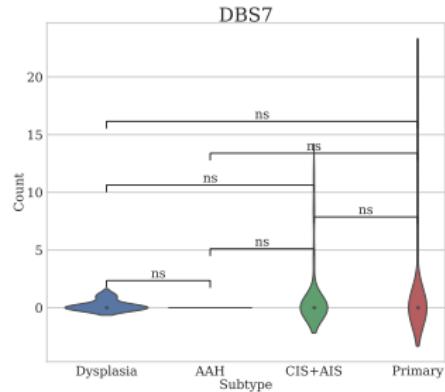
(a) Absolute



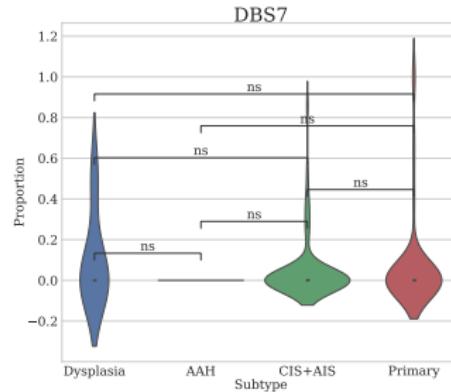
(b) Relative

Figure: DBS2 Signature in LUSC

# DBS in LUAD IV



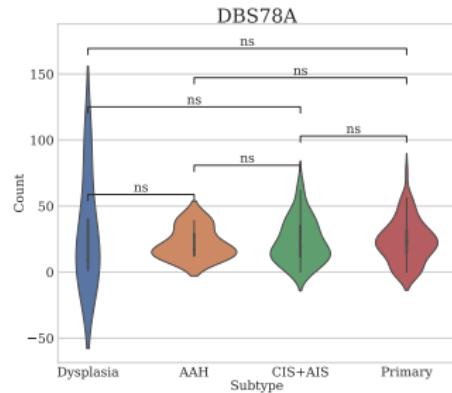
(a) Absolute



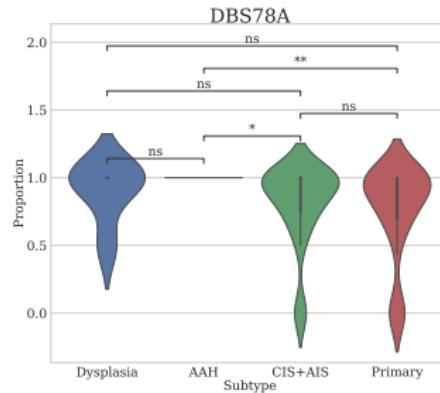
(b) Relative

Figure: DBS7 Signature in LUSC

# DBS in LUAD V



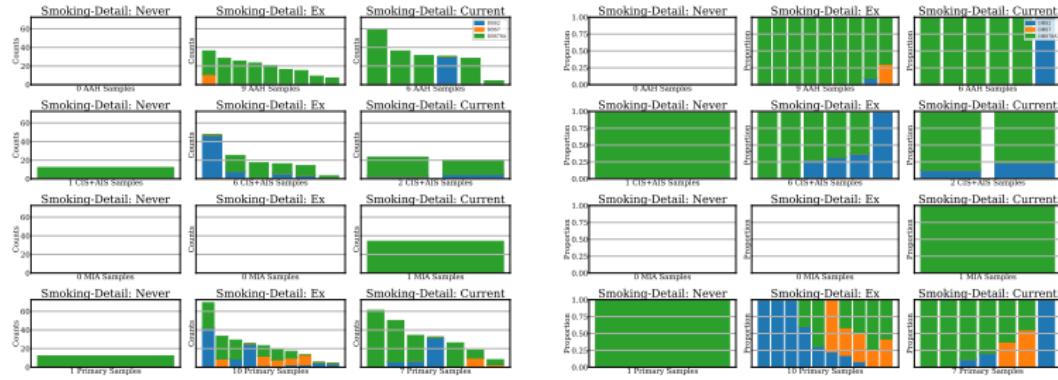
(a) Absolute



(b) Relative

Figure: DBS78A Signature in LUSC

# DBS in LUAD with Smoking I

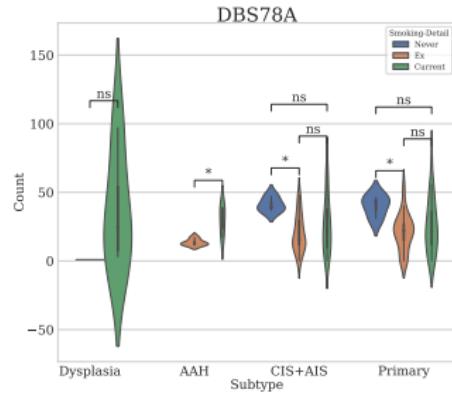


(a) Absolute

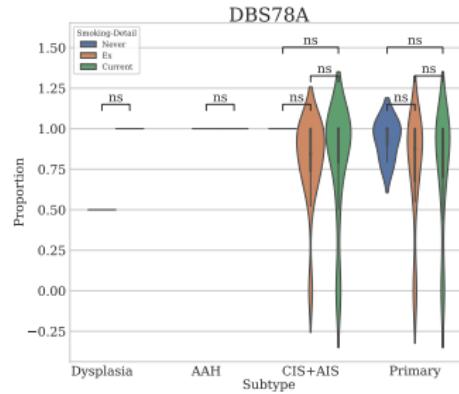
(b) Relative

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

# DBS in LUAD with Smoking II



(a) Absolute



(b) Relative

Figure: DBS78A Signature in LUSC in Smoking

# DBS in LUAD with Recurrence

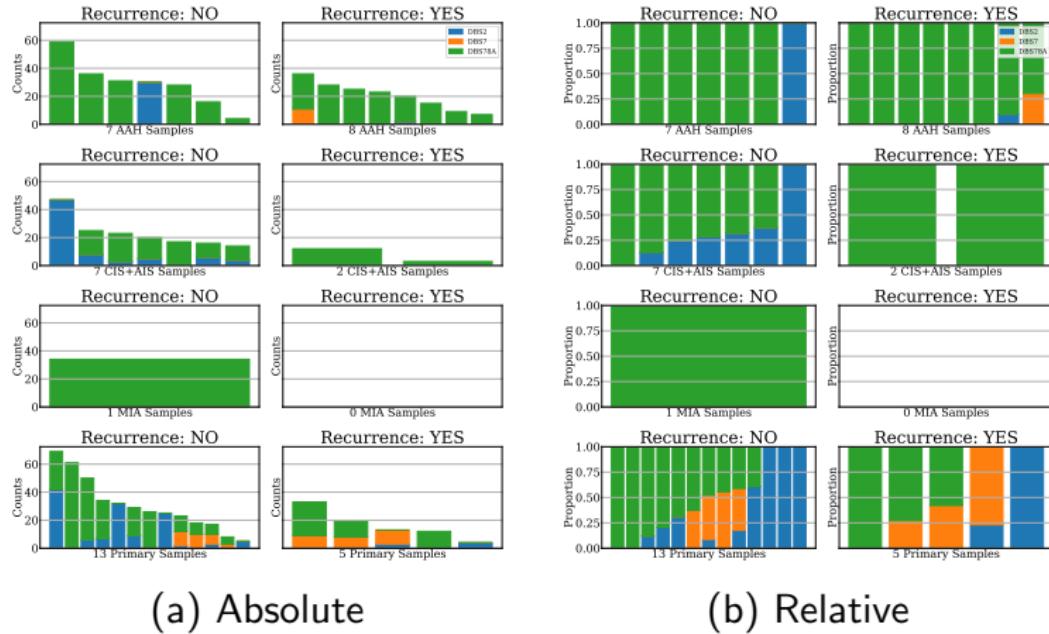


Figure: DBS Bar Plot by Cancer Subtype & Recurrence in LUAD

## 4. Results

### 4.9. Discovery of Mutational Signature

#### 4.9.6. DBS with Shared mutation Proportion in LUSC

# DBS2 with Shared Mutation Proportion in LUSC I

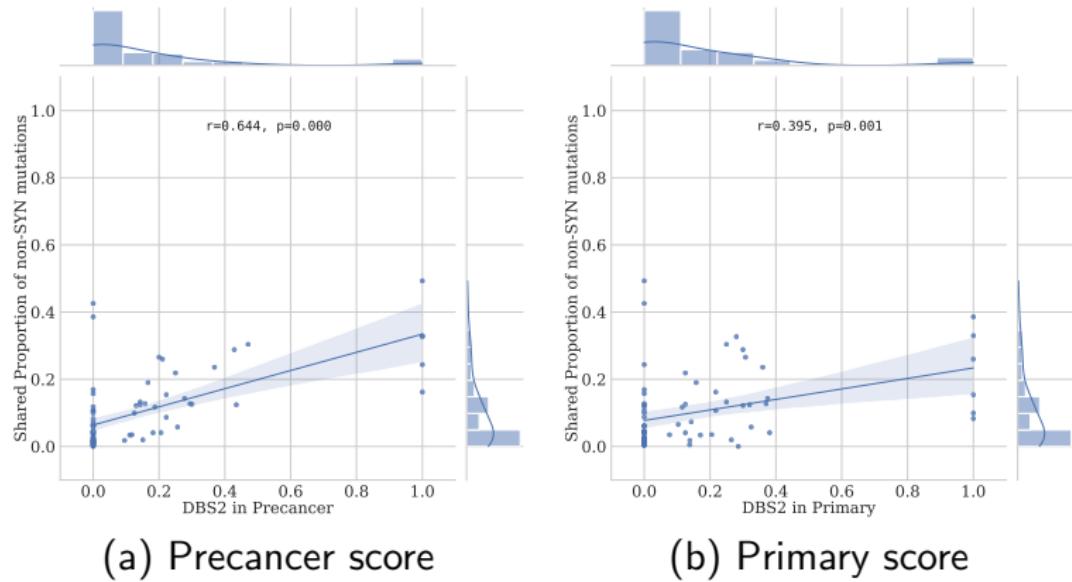
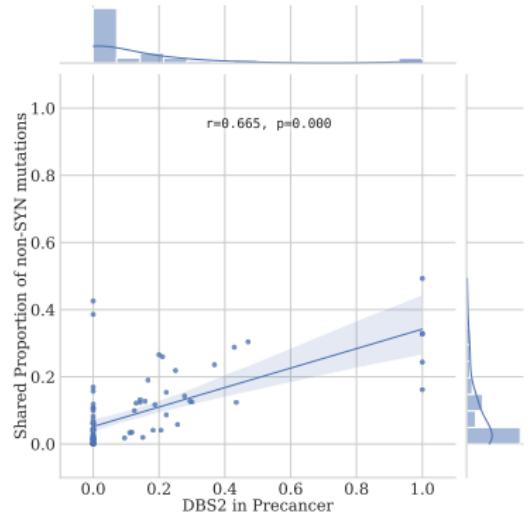
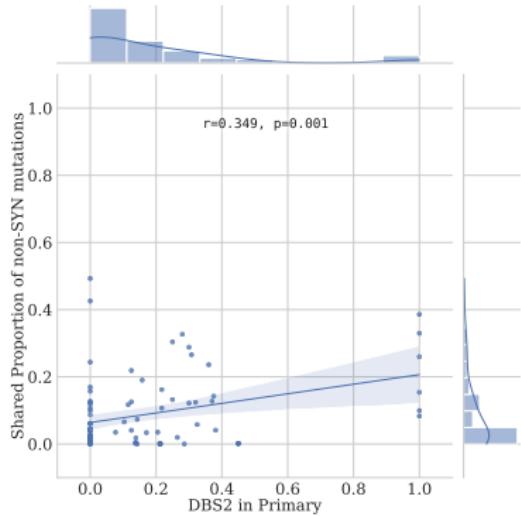


Figure: DBS2 with CIS samples

# DBS2 with Shared Mutation Proportion in LUSC II



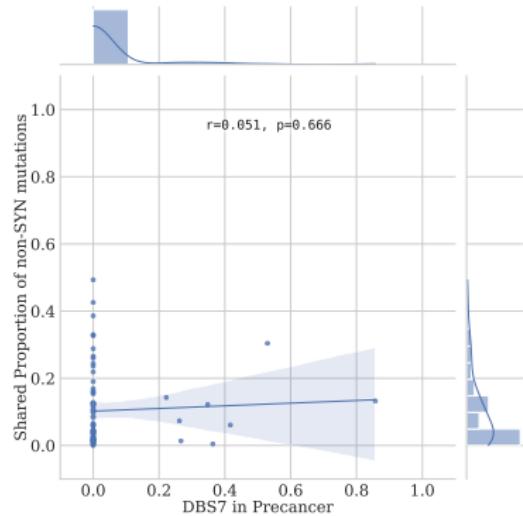
(a) Precancer score



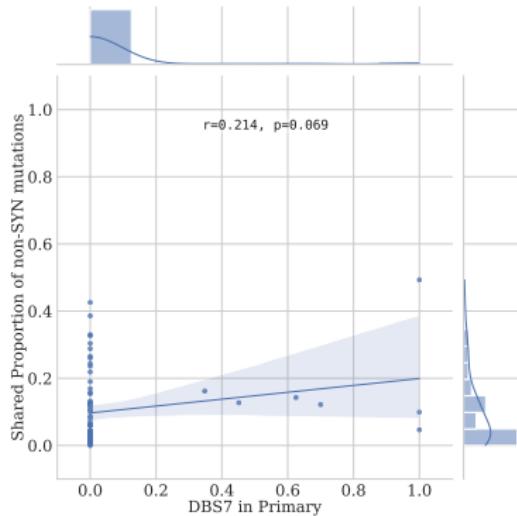
(b) Primary score

Figure: DBS2 with Precancer samples

# DBS7 with Shared Mutation Proportion in LUSC I



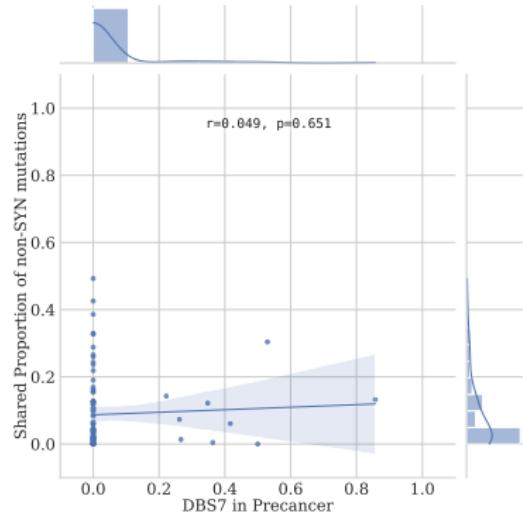
(a) Precancer score



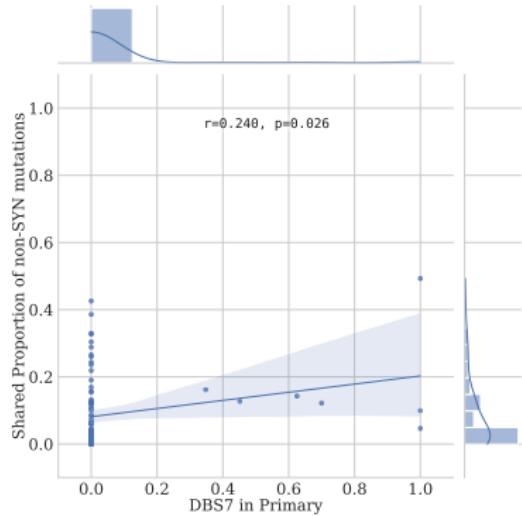
(b) Primary score

Figure: DBS7 with CIS samples

# DBS7 with Shared Mutation Proportion in LUSC II



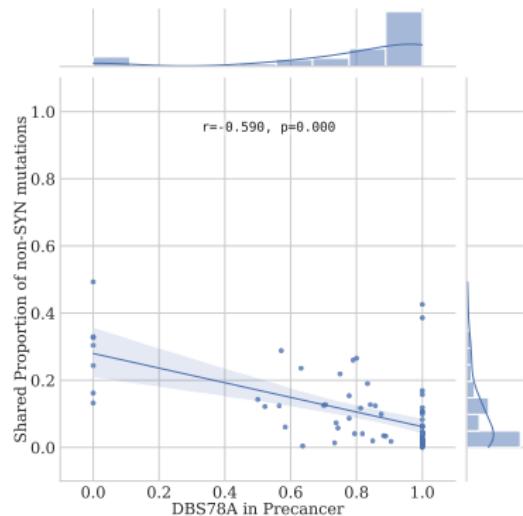
(a) Precancer score



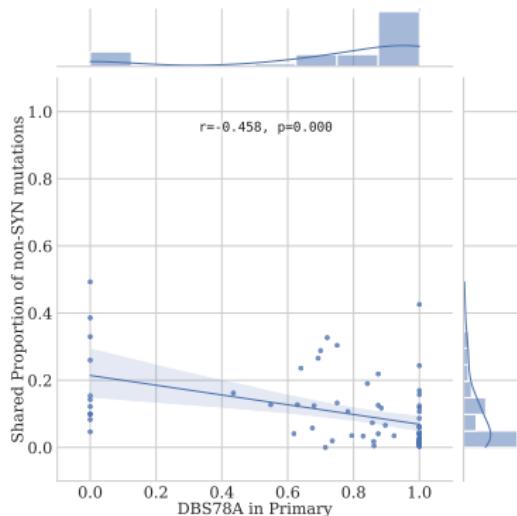
(b) Primary score

Figure: DBS7 with Precancer samples

# DBS78A with Shared Mutation Proportion in LUSC I



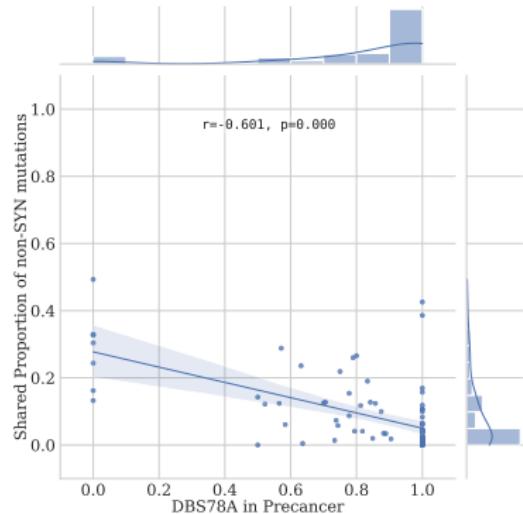
(a) Precancer score



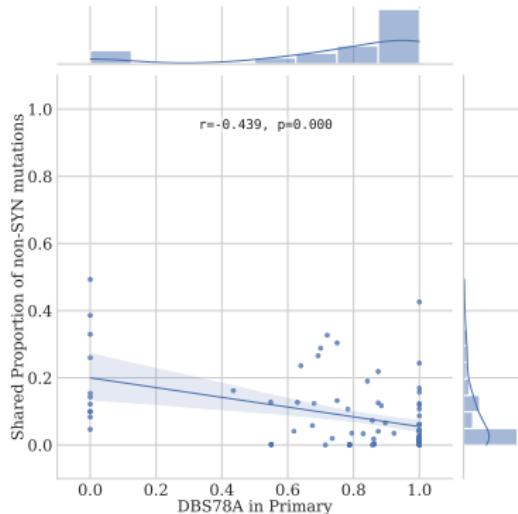
(b) Primary score

Figure: DBS78A with CIS samples

# DBS78A with Shared Mutation Proportion in LUSC II



(a) Precancer score



(b) Primary score

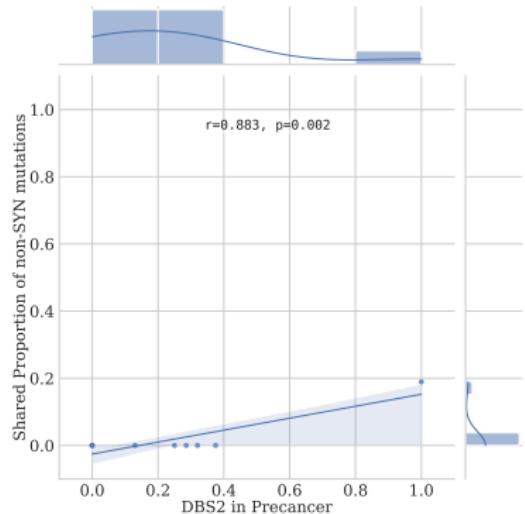
Figure: DBS78A with Precancer samples

## 4. Results

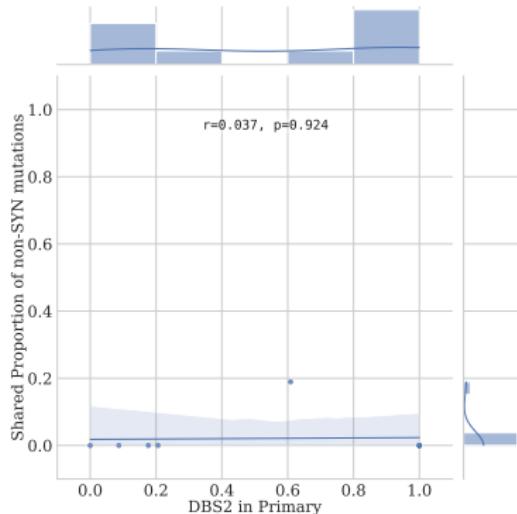
### 4.9. Discovery of Mutational Signature

#### 4.9.7. DBS with Shared Mutation Proportion in LUAD

# DBS2 with Shared Mutation Proportion in LUAD I



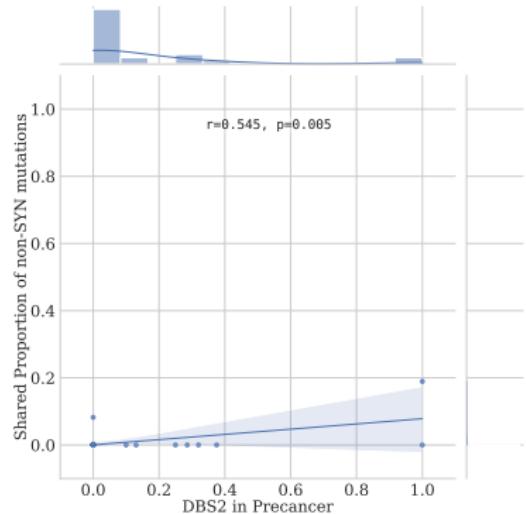
(a) Precancer score



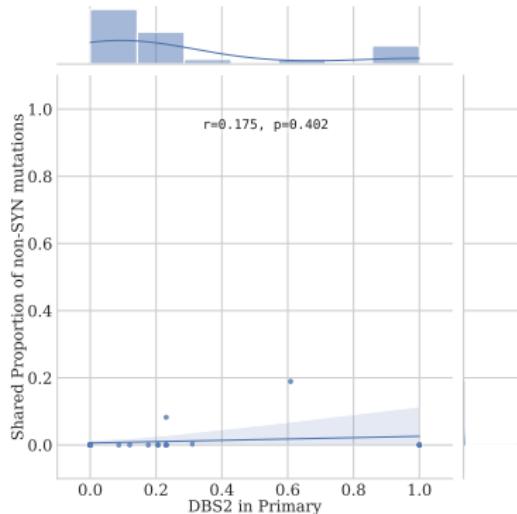
(b) Primary score

Figure: DBS2 with AIS samples

# DBS2 with Shared Mutation Proportion in LUAD II



(a) Precancer score



(b) Primary score

Figure: DBS2 with Precancer samples

## 4. Results

### 4.9. Discovery of Mutational Signature

#### 4.9.8. DBS with Clinical Values in LUSC

# DBS2 with Recurrence-Free Survival in LUSC

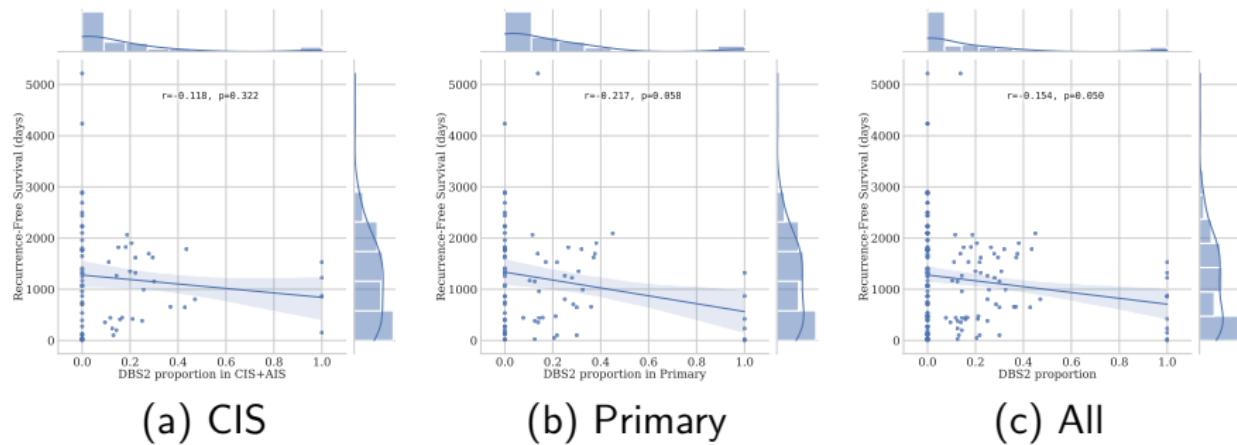


Figure: DBS2 with RFS in LUSC

# DBS78A with Recurrence-Free Survival in LUSC

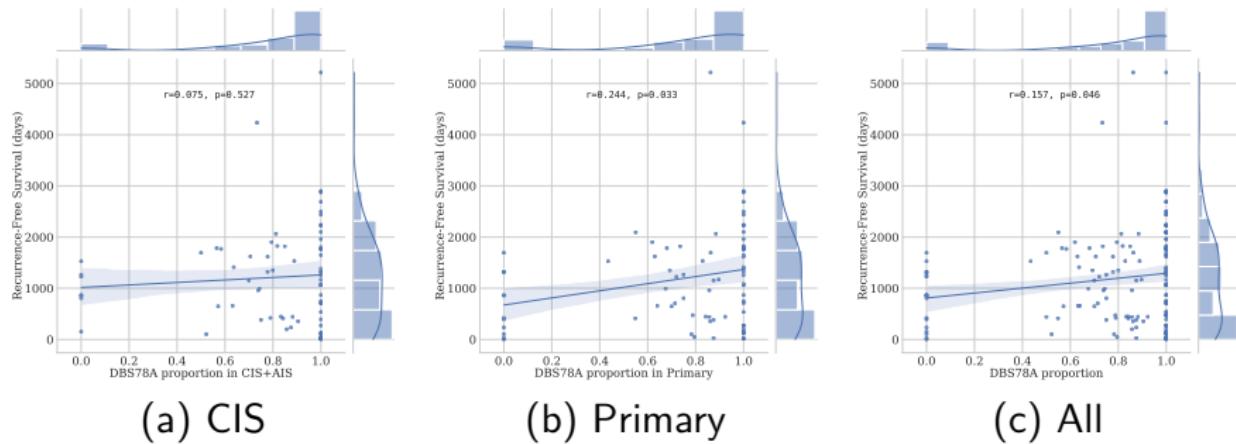
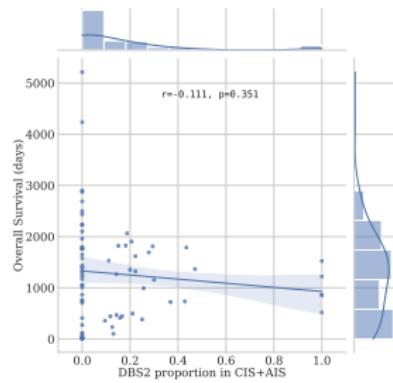
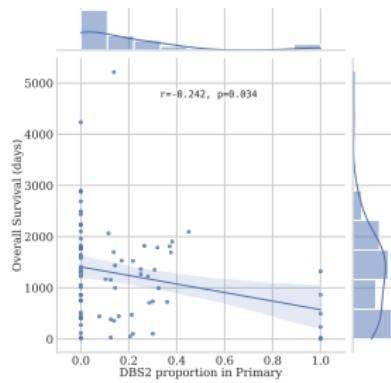


Figure: DBS78A with RFS in LUSC

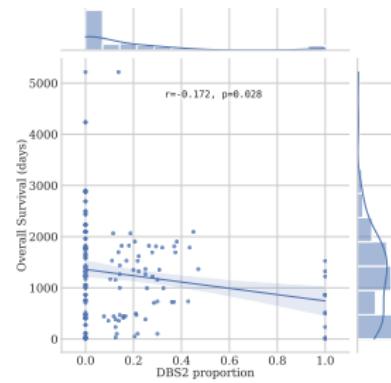
# DBS2 with Overall Survival in LUSC I



(a) CIS



(b) Primary



(c) All

Figure: DBS2 with RFS in LUSC

## 4. Results

### 4.9. Discovery of Mutational Signature

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

# Indel signatures I

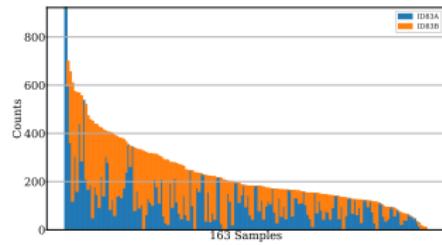
ID83A

- Unknown

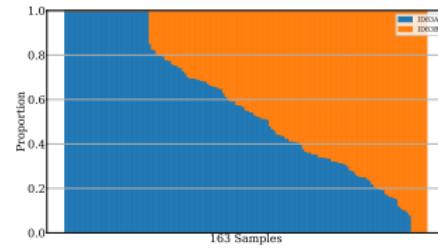
ID83B

- Unknown

# Indels in LUSC I



(a) Absolute



(b) Relative

Figure: Indel Bar Plot in LUSC

# Indels in LUSC II

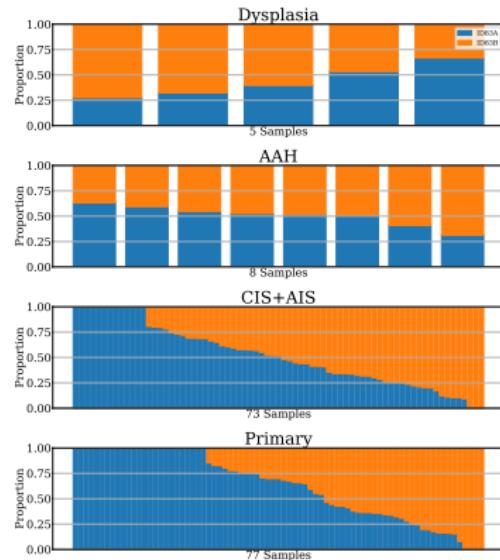
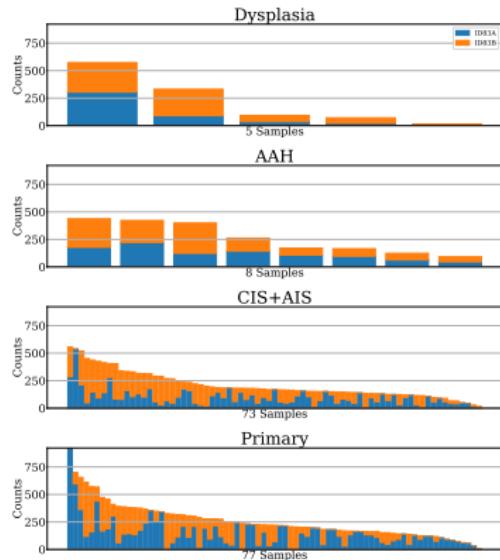
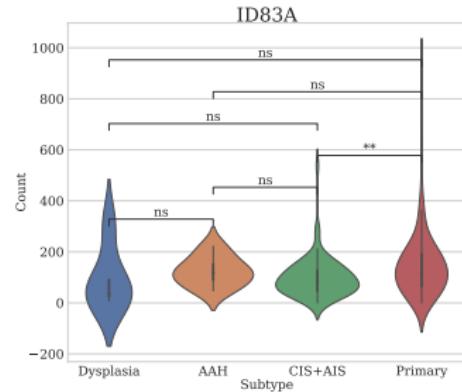
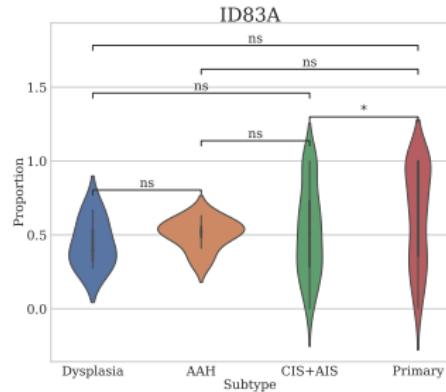


Figure: Indel Bar Plot by Cancer Subtype in LUSC

# Indels in LUSC III



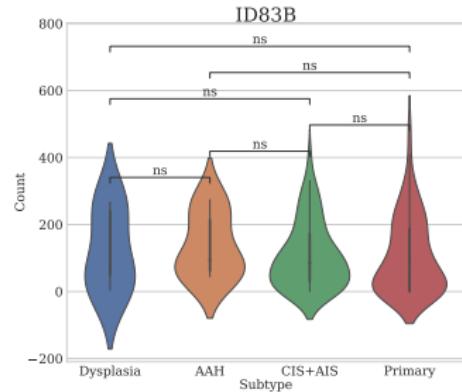
(a) Absolute



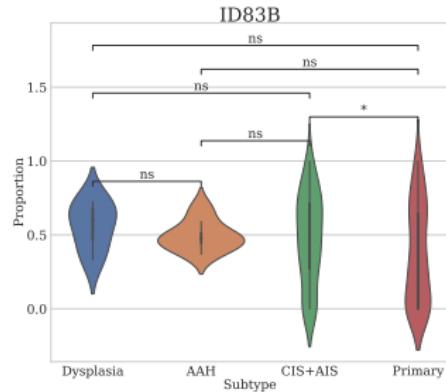
(b) Relative

Figure: Indel83A Signature in LUSC

# Indels in LUSC IV



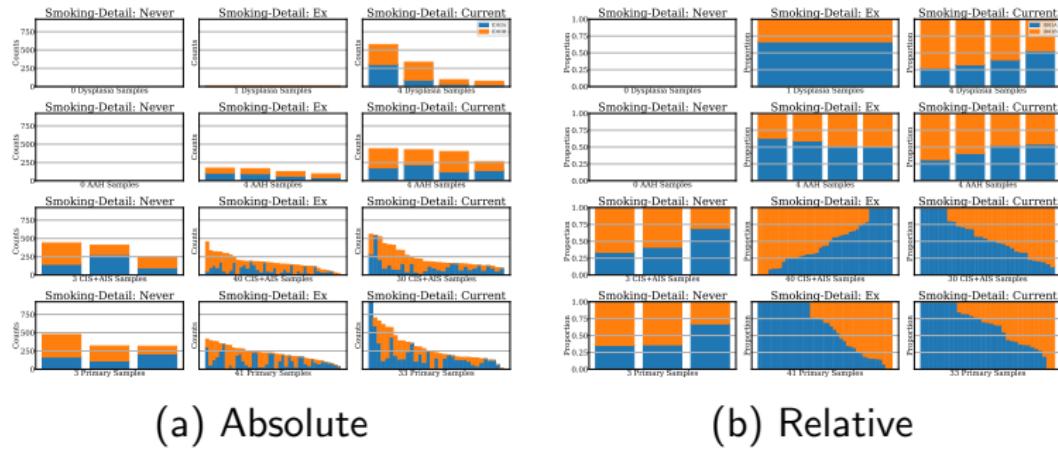
(a) Absolute



(b) Relative

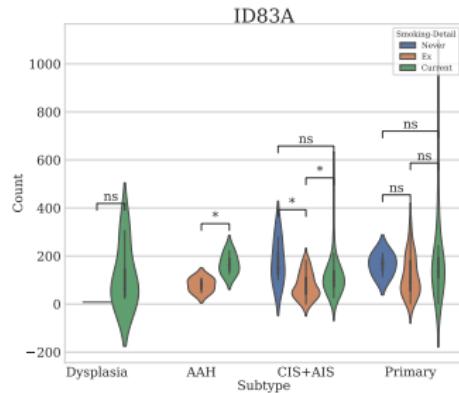
Figure: Indel83B Signature in LUSC

# Indel in LUSC with Smoking I

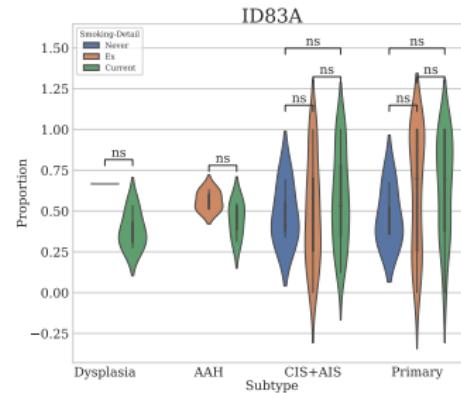


**Figure:** Indel Bar Plot by Cancer Subtype & Smoking in LUSC

# Indel in LUSC with Smoking II



(a) Absolute



(b) Relative

Figure: Indel83A Signature in LUSC with Smoking

# Indel in LUSC with Recurrence

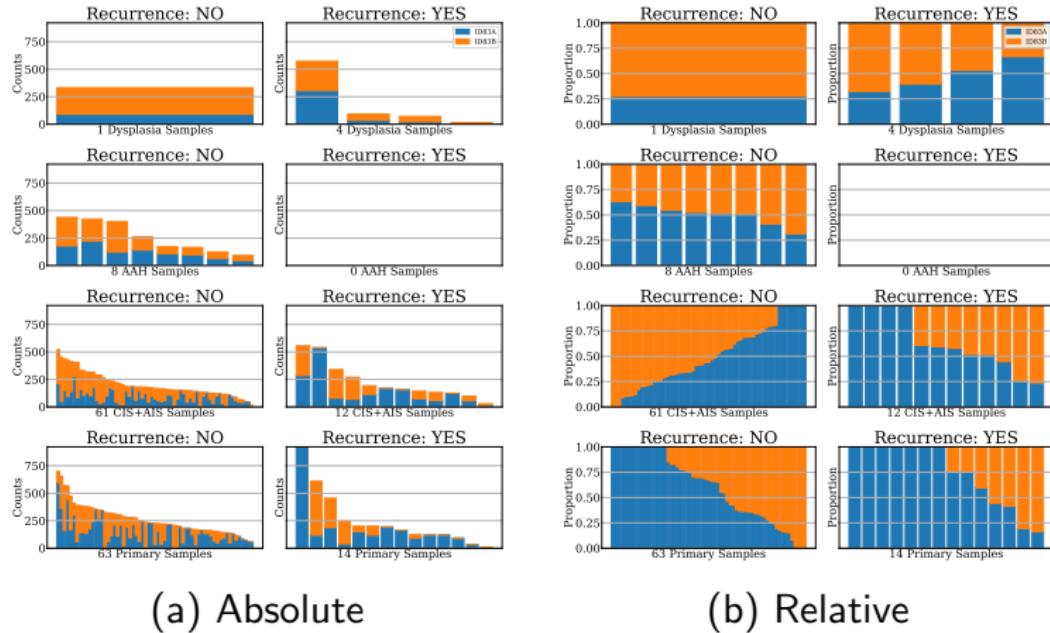
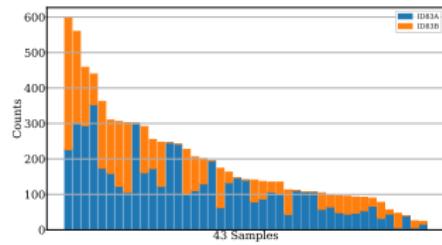
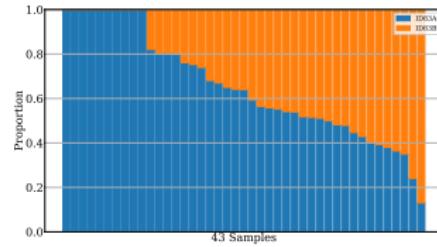


Figure: Indel Bar Plot by Cancer Subtype & Recurrence in LUSC

# Indels in LUAD I



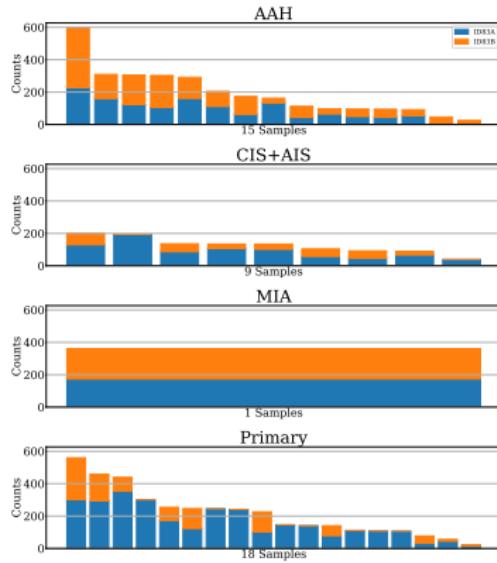
(a) Absolute



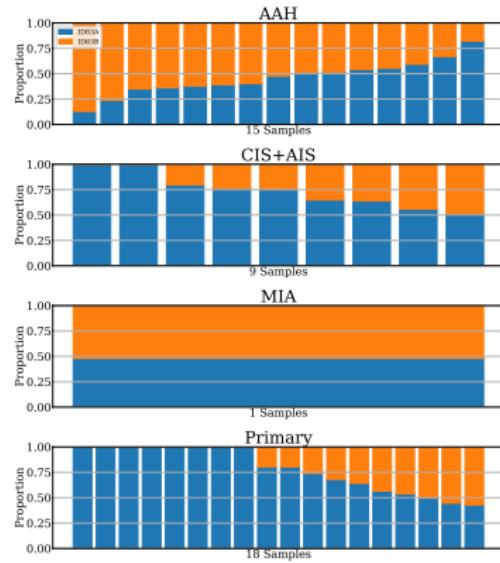
(b) Relative

Figure: Indel Bar Plot in LUAD

# Indels in LUAD II



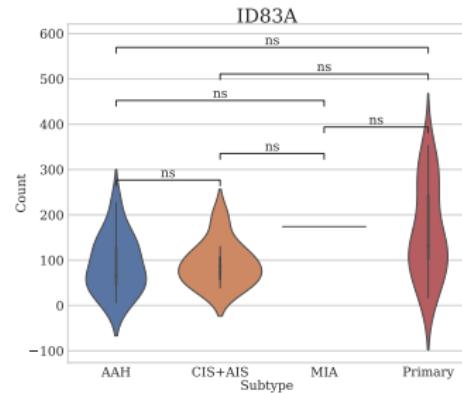
(a) Absolute



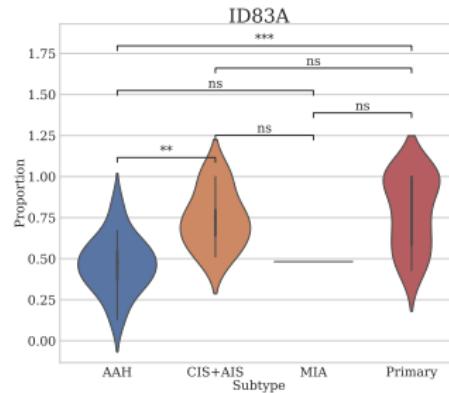
(b) Relative

Figure: Indel Bar Plot by Cancer Subtype in LUAD

# Indels in LUAD III



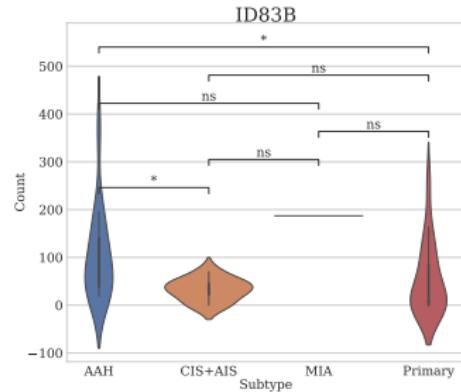
(a) Absolute



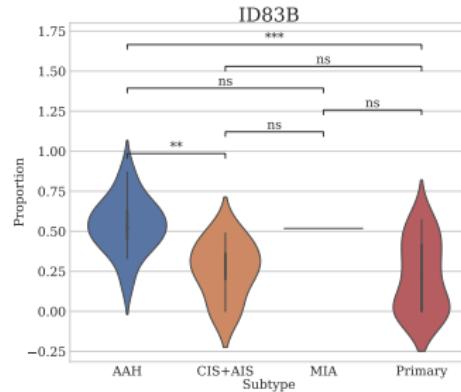
(b) Relative

Figure: Indel83A Signature in LUAD

# Indels in LUAD IV



(a) Absolute



(b) Relative

Figure: Indel83B Signature in LUAD

# Indel in LUAD with Smoking

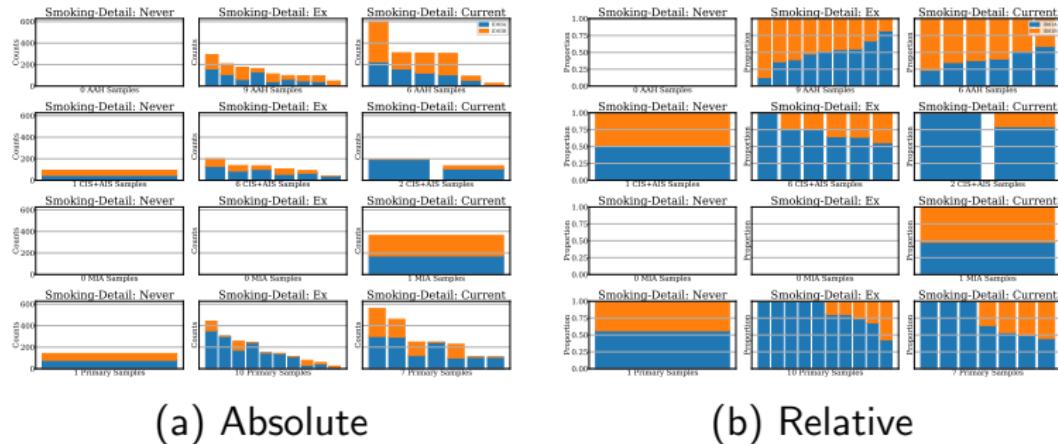


Figure: Indel Bar Plot by Cancer Subtype & Smoking in LUAD

# Indel in LUAD with Recurrence

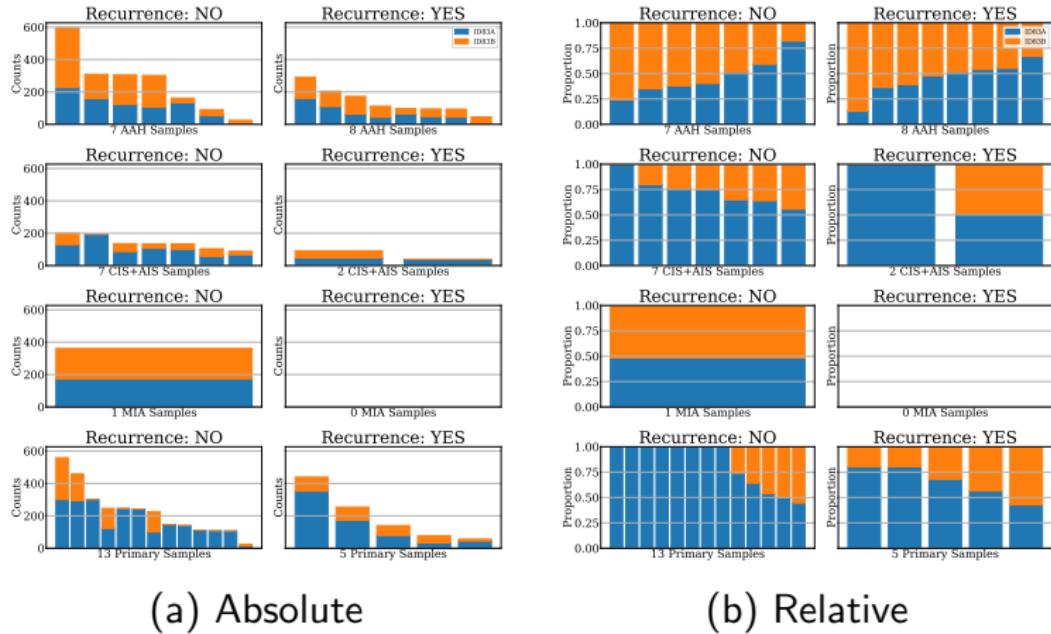


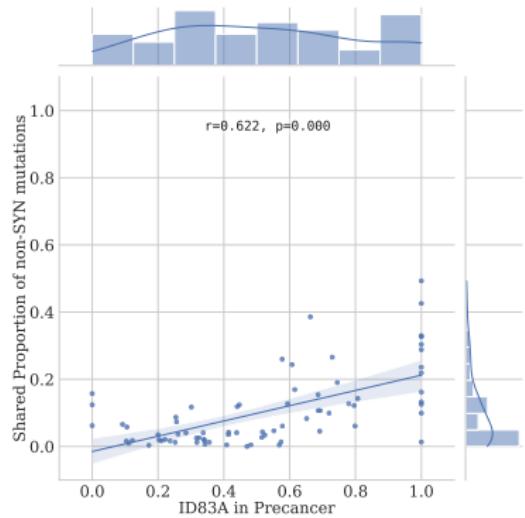
Figure: Indel Bar Plot by Cancer Subtype & Recurrence in LUAD

## 4. Results

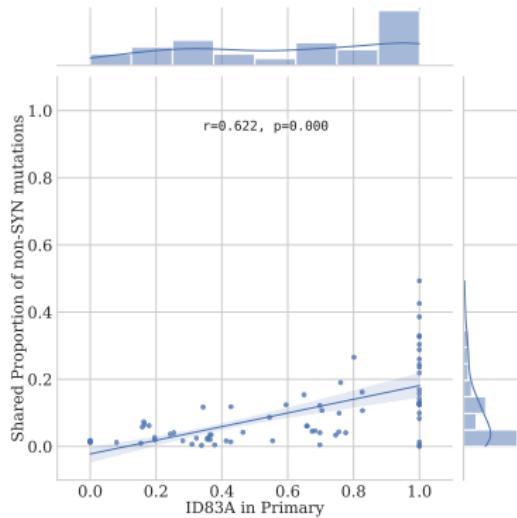
### 4.9. Discovery of Mutational Signature

#### 4.9.10. Indel with Shared mutation Proportion in LUSC

# ID83A with Shared Mutation Proportion in LUSC I



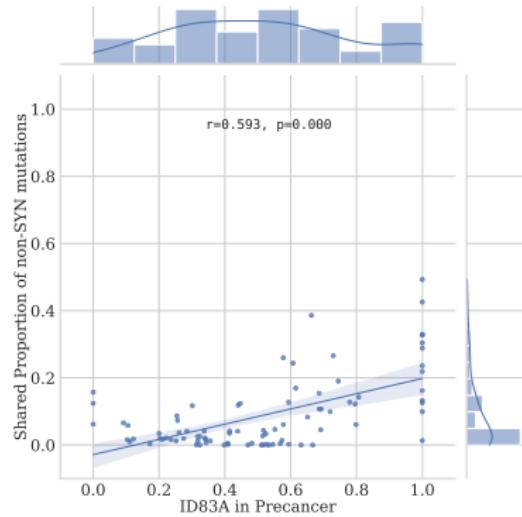
(a) Precancer score



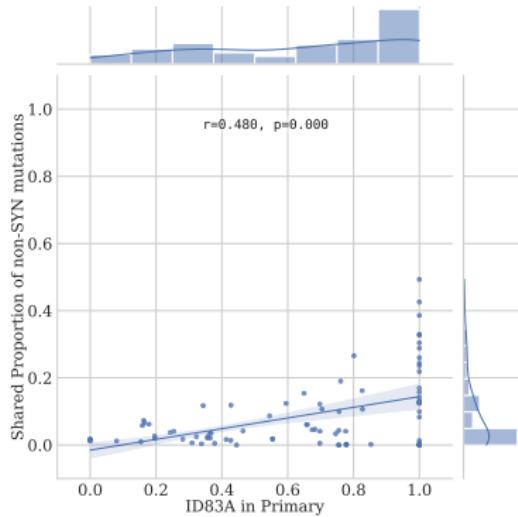
(b) Primary score

Figure: ID83A with CIS samples

# ID83A with Shared Mutation Proportion in LUSC II



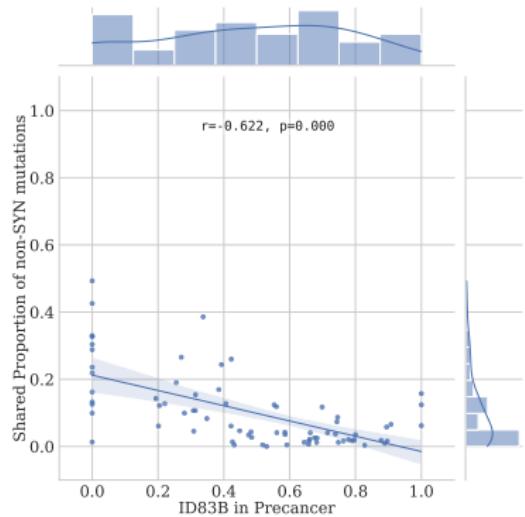
(a) Precancer score



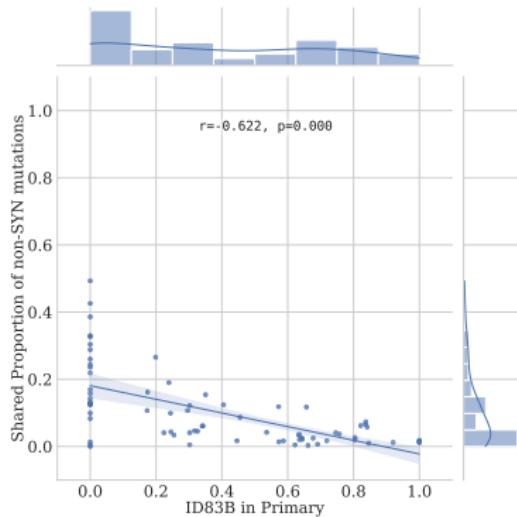
(b) Primary score

Figure: ID83A with Precancer samples

# ID83B with Shared Mutation Proportion in LUSC I



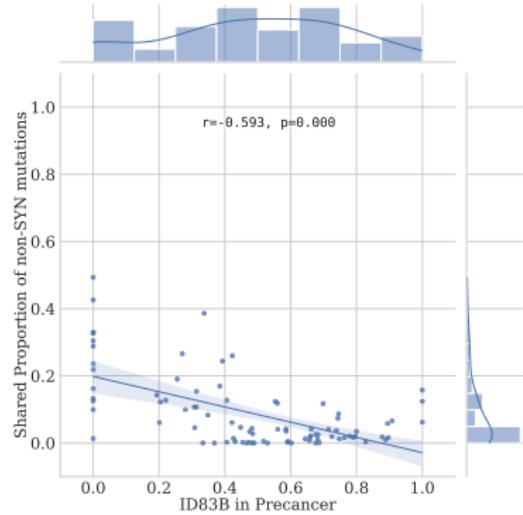
(a) Precancer score



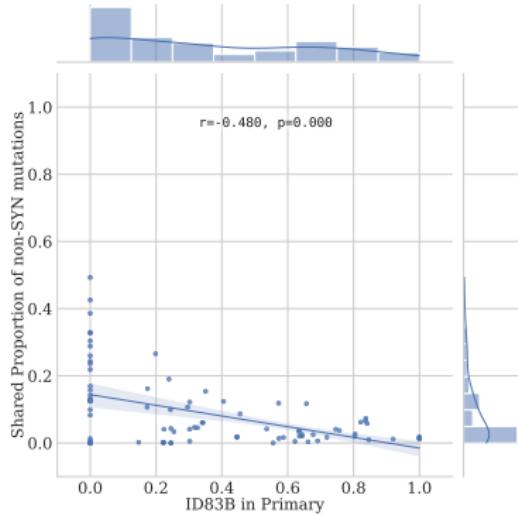
(b) Primary score

Figure: ID83B with CIS samples

# ID83B with Shared Mutation Proportion in LUSC II



(a) Precancer score



(b) Primary score

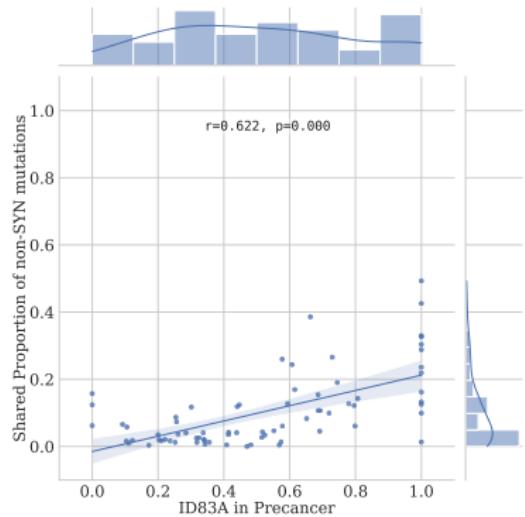
Figure: ID83B with Precancer samples

## 4. Results

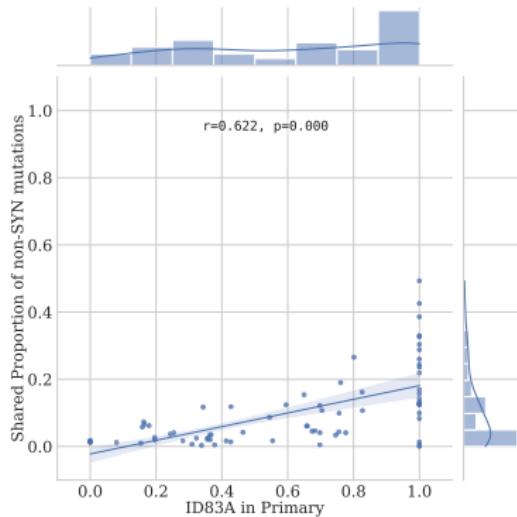
### 4.9. Discovery of Mutational Signature

#### 4.9.11. Indel with Shared Mutation Proportion in LUSC

# ID83A with Shared Mutation Proportion in LUSC I



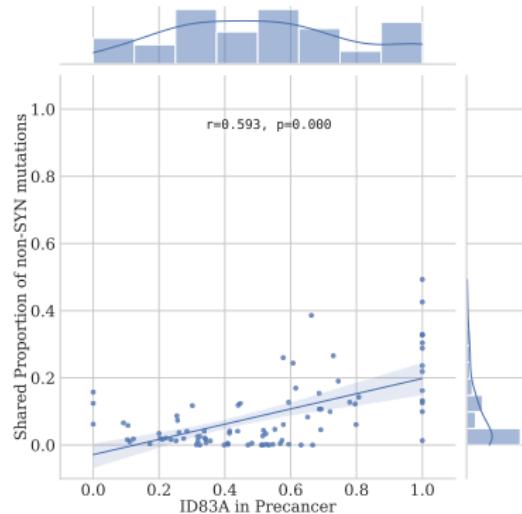
(a) Precancer score



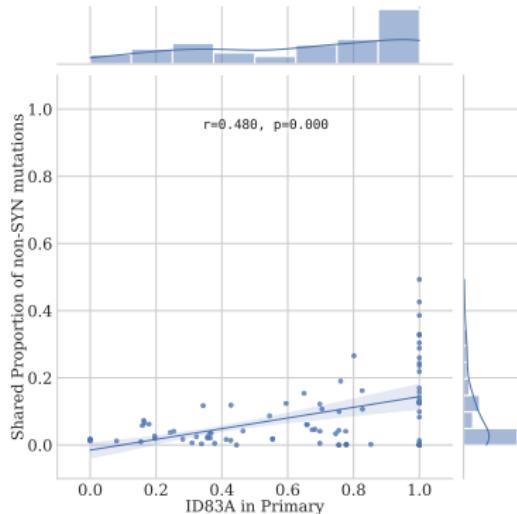
(b) Primary

Figure: ID83A with CIS samples

# ID83A with Shared Mutation Proportion in LUSC II



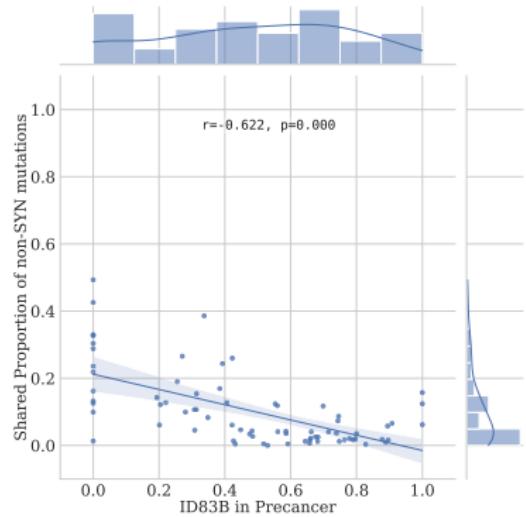
(a) Precancer score



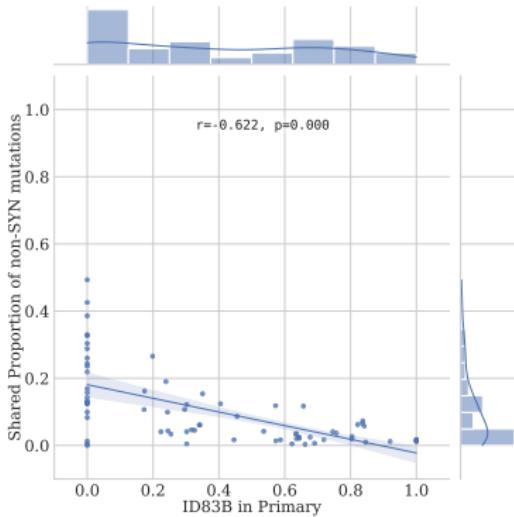
(b) Primary

Figure: ID83A with Precacner samples

# ID83B with Shared Mutation Proportion in LUSC I



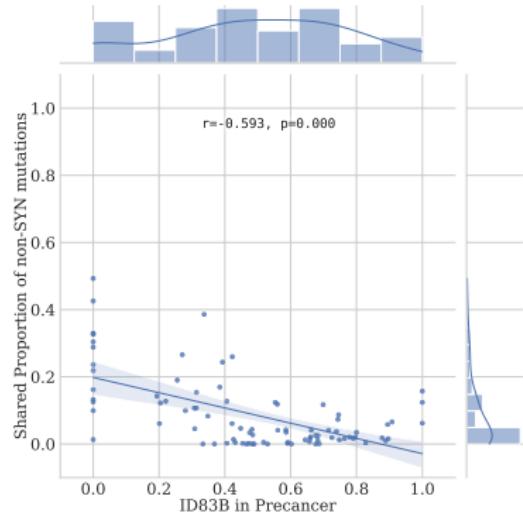
(a) Precancer score



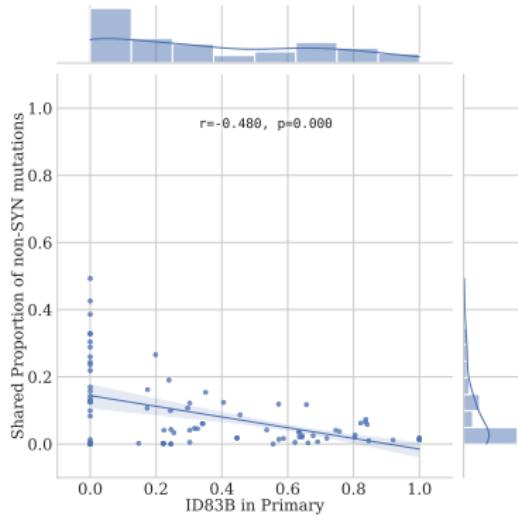
(b) Primary

Figure: ID83B with CIS samples

# ID83B with Shared Mutation Proportion in LUSC II



(a) Precancer score



(b) Primary

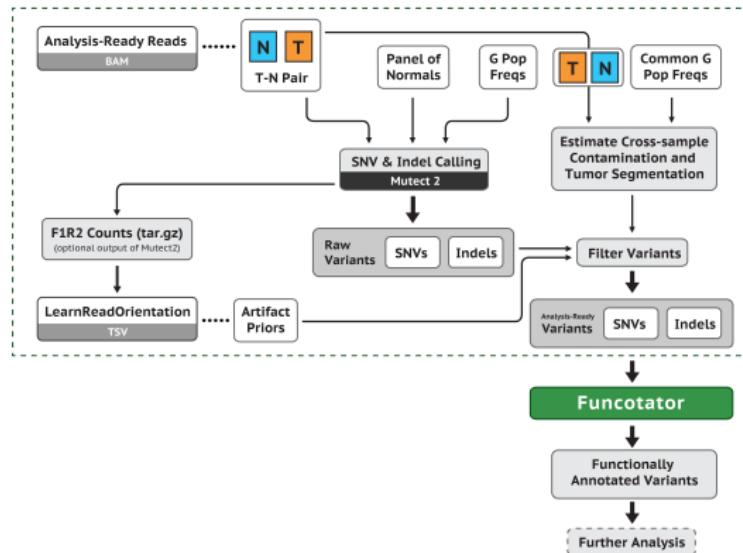
Figure: ID83B with Precancer samples

# Findings in Mutation Signature

## 4. Results

### 4.10. Clinical Data with Point Mutation

# Mutect2?



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

## 4. Results

### 4.10. Clinical Data with Point Mutation

#### 4.10.1. 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

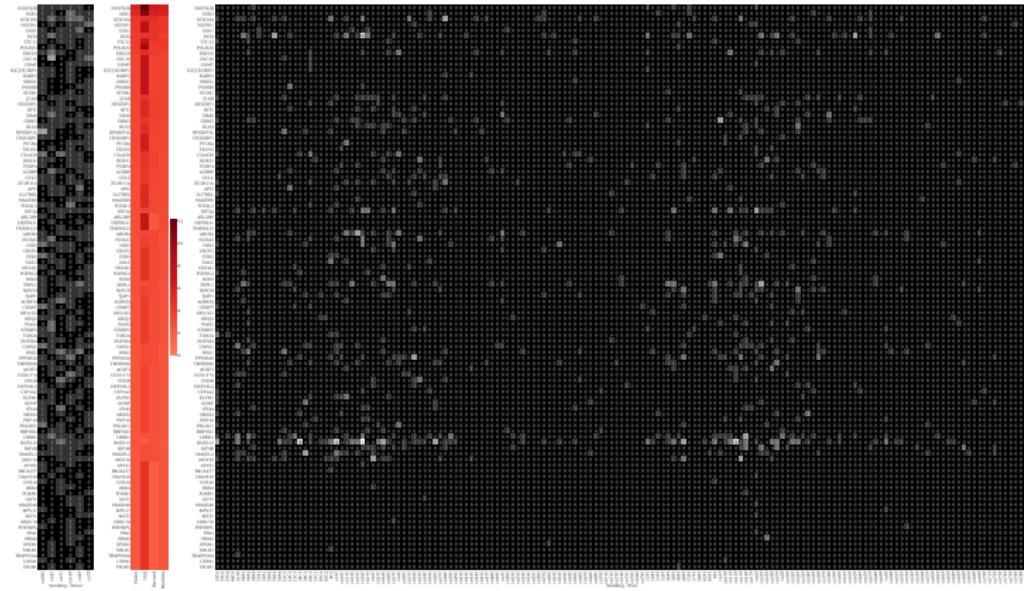


Figure: Clinical Data about LUSC for Smoking

# Clinical Data about LUSC for Smoking II

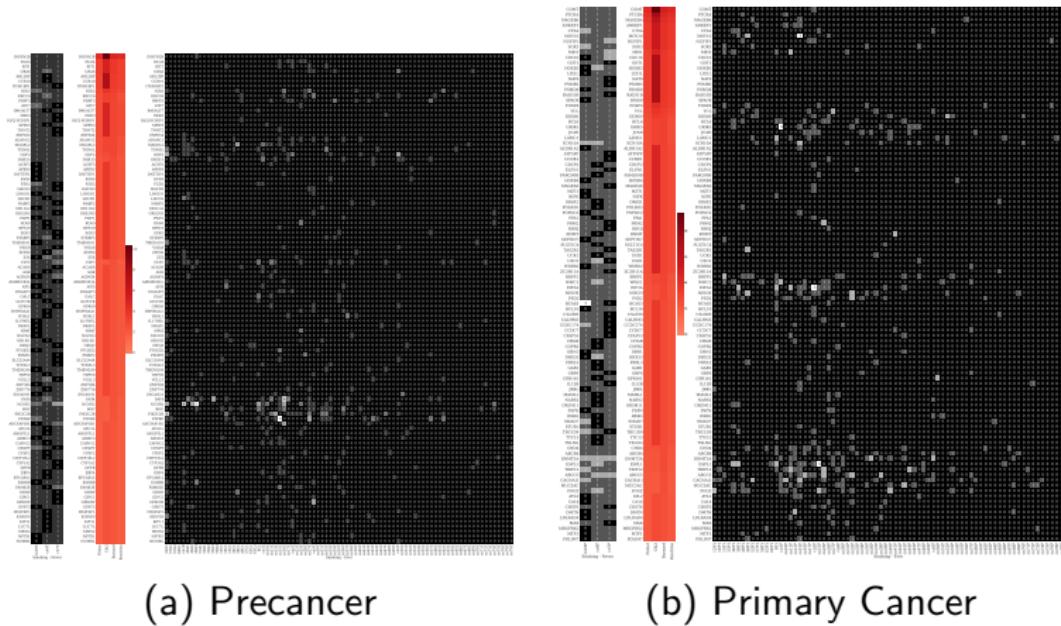


Figure: Clinical Data about LUSC for Smoking with Precancer/Primary

# 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).

# 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

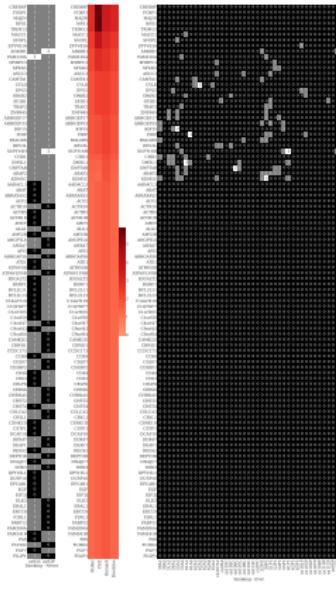
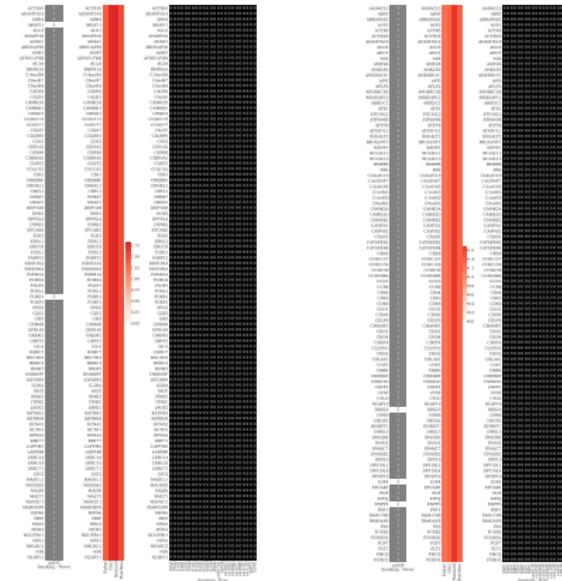


Figure: Clinical Data about LUAD for Smoking

## 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

### 4.10. Clinical Data with Point Mutation

#### 4.10.2. 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

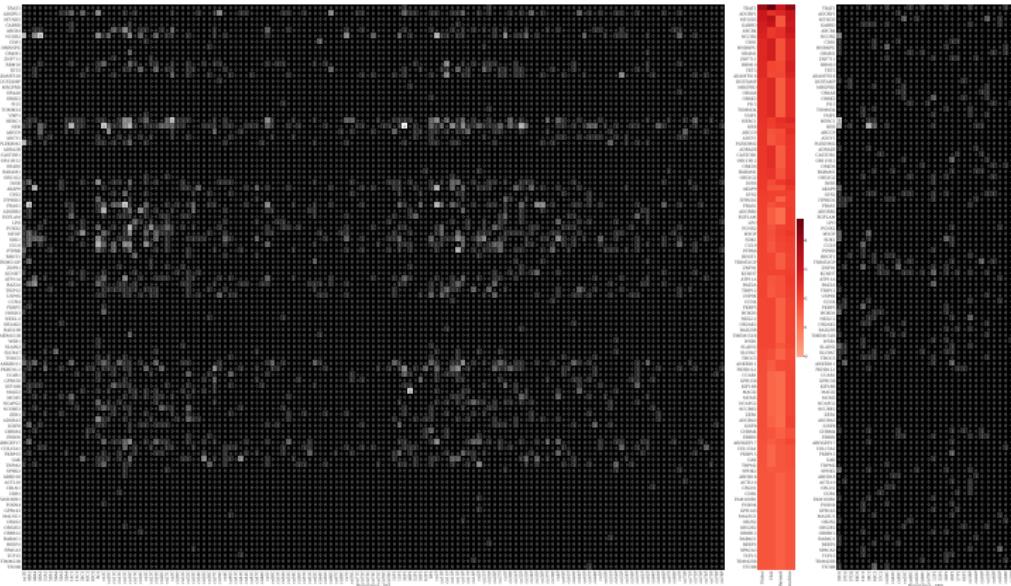


Figure: Clinical Data about LUSC for Recurrence

# Clinical Data about LUSC for Recurrence II

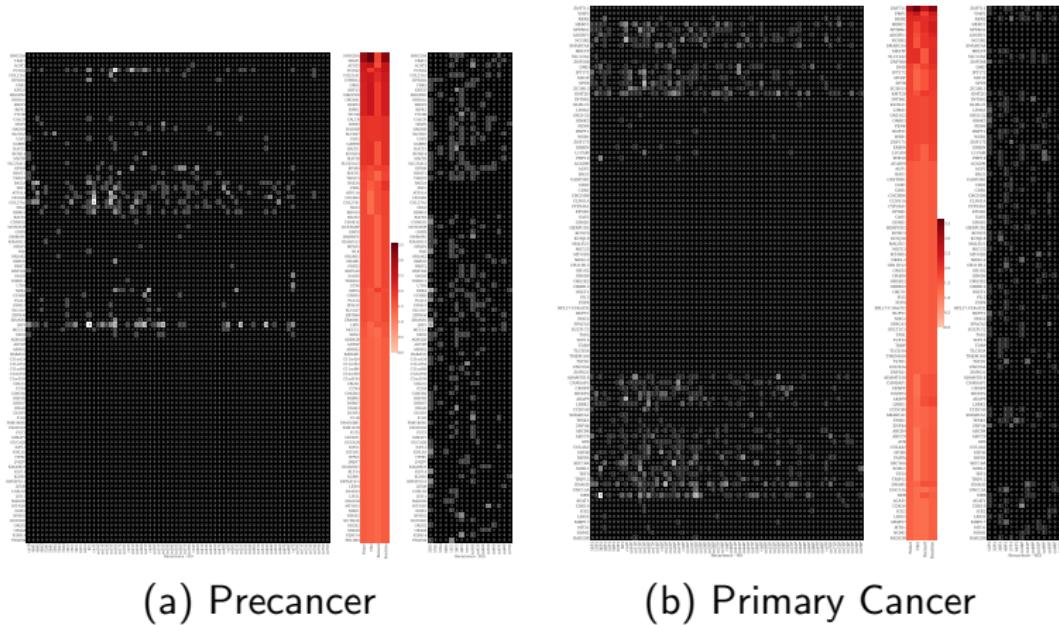


Figure: Clinical Data about LUSC for Recurrence with Precancer/Primary

# 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

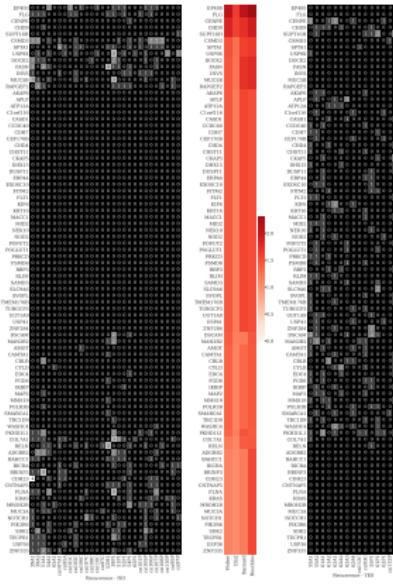
- ① ZNF711 is the best indicator for Recurrence in Primary.

# LUAD with Recurrence

Table: LUAD WES Data with Recurrence

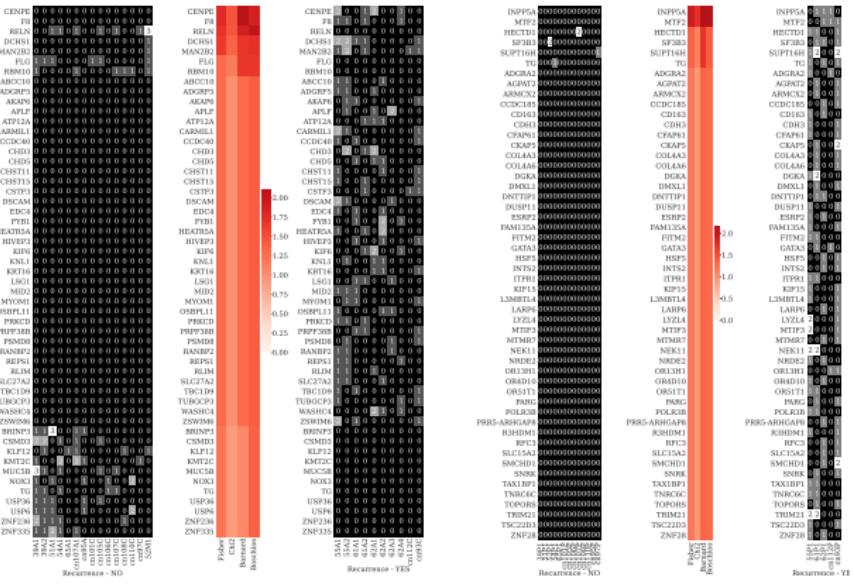
Recurrence?	Stage	Number of Samples	
		Normal	Affected
Recurrence	Normal	5	5
	AAH	8	8
	CIS+AIS	2	2
	Primary	5	5
	Total	20	20
Non-recurrence	Normal	13	13
	AAH	7	7
	CIS+AIS	7	7
	MIA	1	1
	Primary	13	13
	Total	41	41

## 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

### 4.11. Differences in Gene Expression Levels

# RSEM?

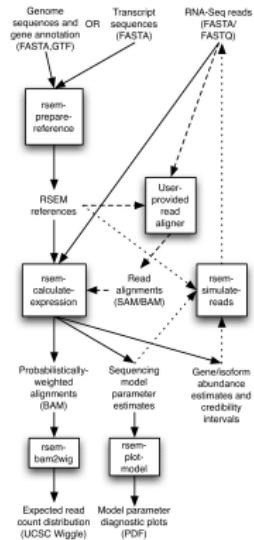


Figure: RSEM workflow (B. Li & Dewey, 2011)

# DESeq2?

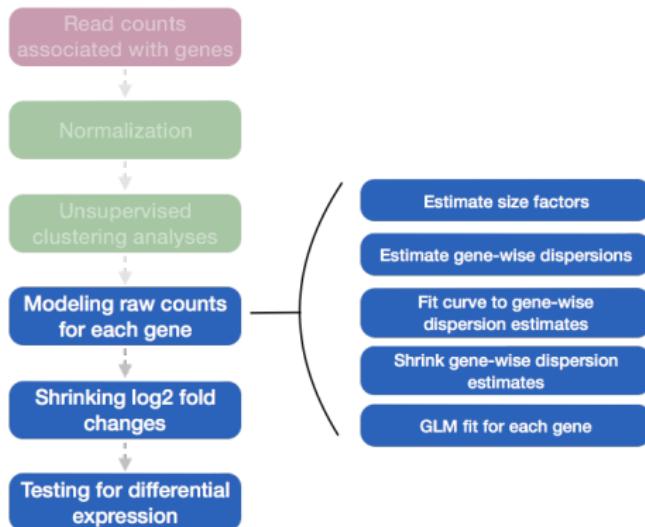


Figure: DESeq2 workflow (Love, Huber, & Anders, 2014)

# 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?

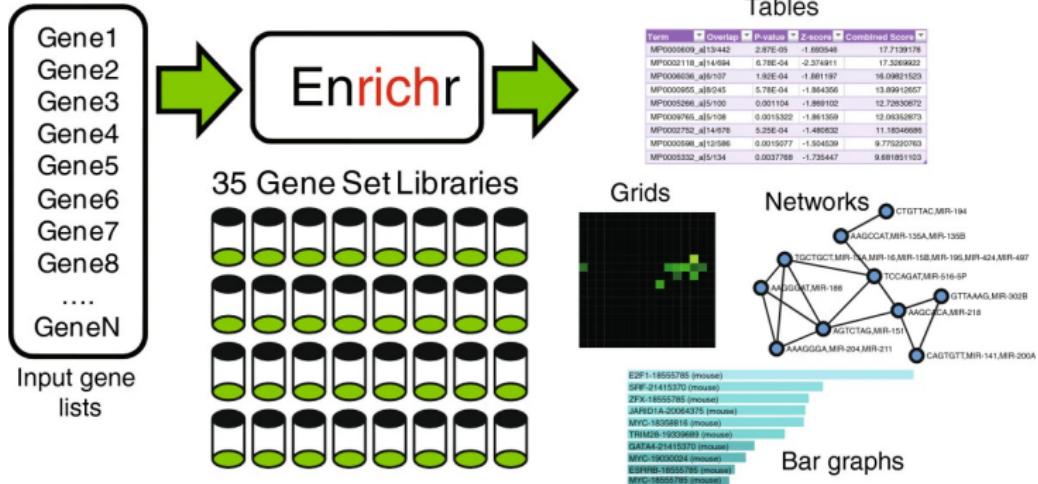


Figure: Enrichr workflow (E. Y. Chen et al., 2013; Kuleshov et al., 2016)

# Gene-set Library

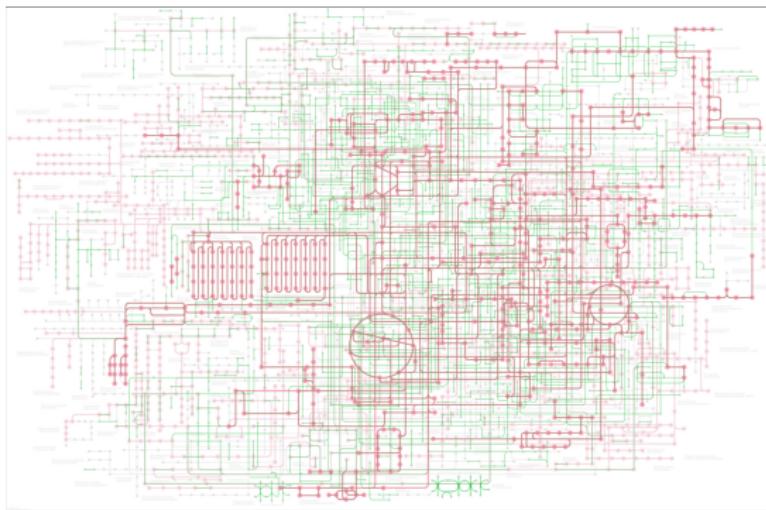


Figure: The global map of metabolic pathways by KEGG (Kanehisa et al., 2021)

KEGG

KEGG 2021 Human

# WTS Data Composition

Table: Number of WTS samples

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

# WTS Data Composition by Recur |

Table: Number of WTS LUSC samples

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

# WTS Data Composition by Recur II

Table: Number of WTS LUAD samples

Recurrence?	Stage	Number of Samples	
		Normal	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	

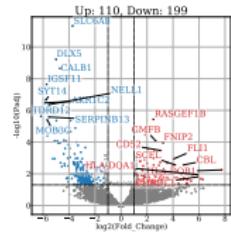
## 4. Results

### 4.11. Differences in Gene Expression Levels

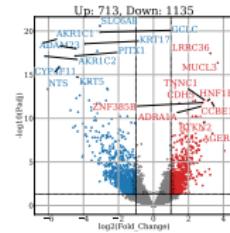
#### 4.11.1. Comparing cancer stage in LUSC

# DEG Volcano Plots in LUSC

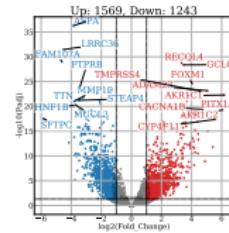
Normal → Dysplasia → CIS → Primary (LUSC)



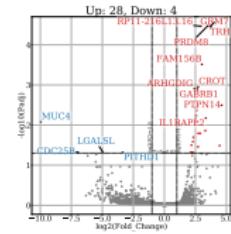
(a) Normal-Dysplasia



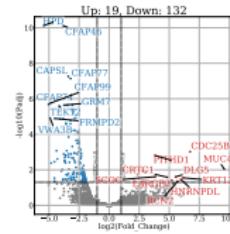
(b) Normal-CIS



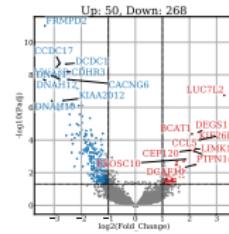
(c) Normal-Primary



(d) Dysplasia-CIS



(e) Dysplasia-Primary

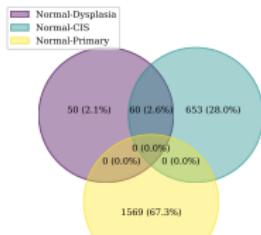


(f) CIS-Primary

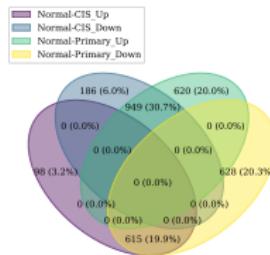
Figure: DEG Volcano Plots in LUSC

# DEG Venn Diagram in LUSC

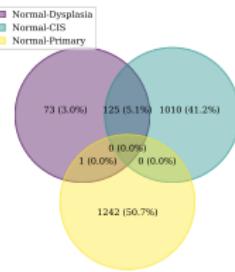
Normal → Dysplasia → CIS → Primary (LUSC)



(a) Up-regulated



(b) Both



(c) Down-regulated

Figure: DEG Venn Diagram in LUSC

# Enrichment test with Normal vs. Dysplasia in LUSC

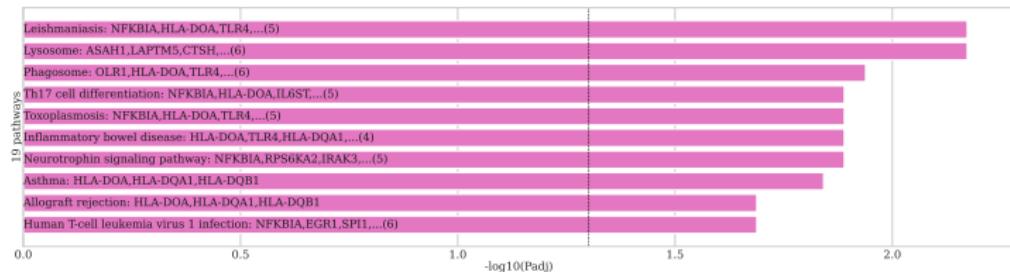


Figure: Up-regulated Pathways on Normal vs. Dysplasia



Figure: Down-regulated Pathways on Normal vs. Dysplasia

# Enrichment test with Normal vs. CIS in LUSC

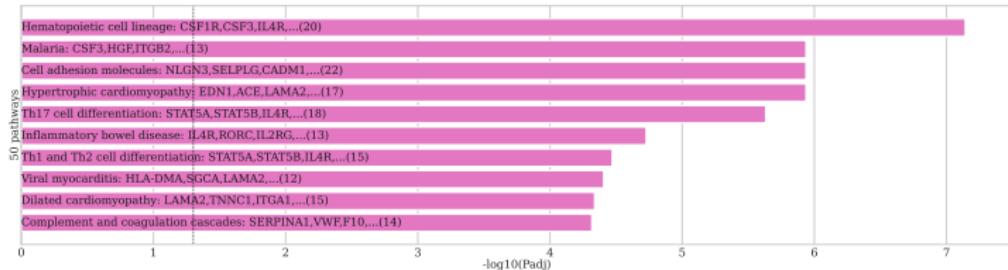


Figure: Up-regulated Pathways on Normal vs. CIS

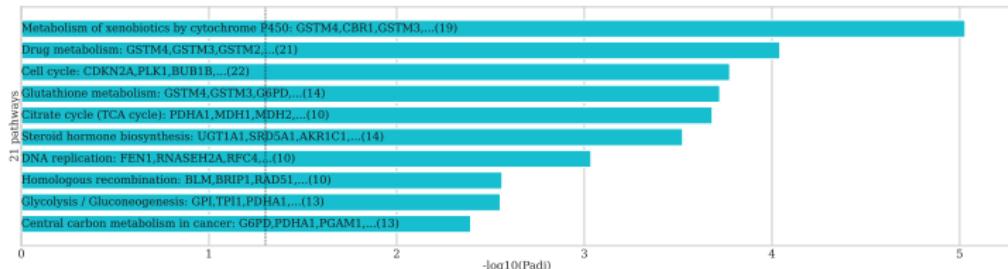


Figure: Down-regulated Pathways on Normal vs. CIS

# Enrichment test with Normal vs. Primary in LUSC

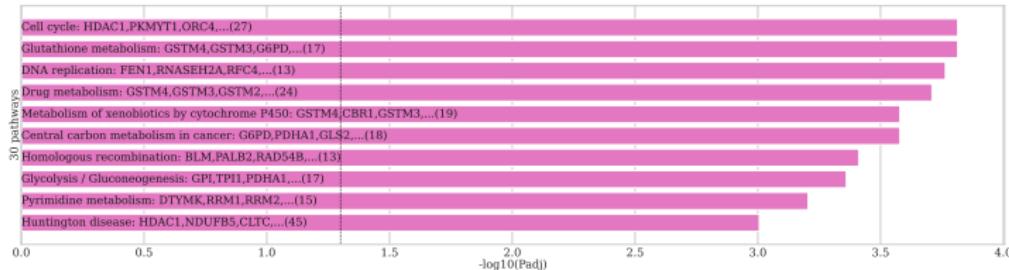


Figure: Up-regulated Pathways on Normal vs. Primary

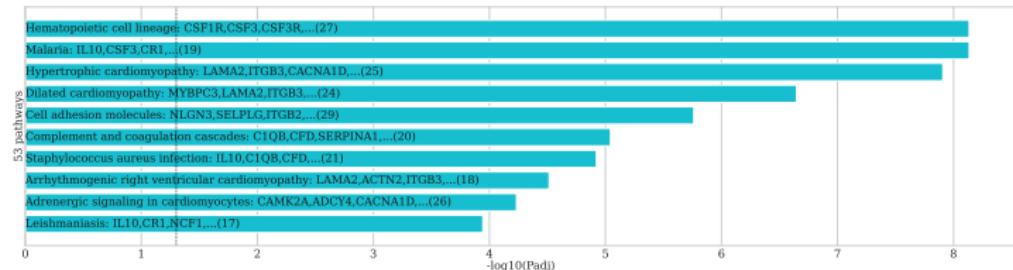


Figure: Down-regulated Pathways on Normal vs. Primary

# 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).

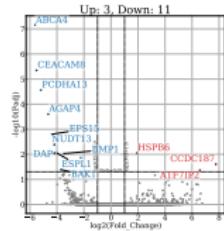
## 4. Results

### 4.11. Differences in Gene Expression Levels

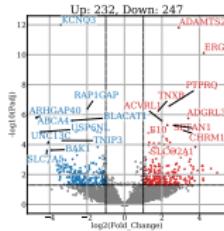
#### 4.11.2. Comparing cancer stage in LUAD

# DEG Volcano Plots in LUAD

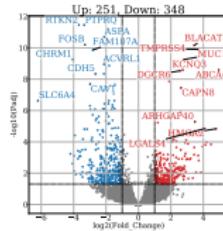
Normal → AAH → AIS → Primary (LUAD)



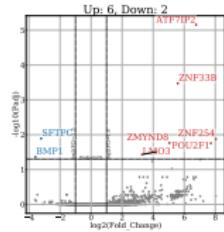
(a) Normal-AAH



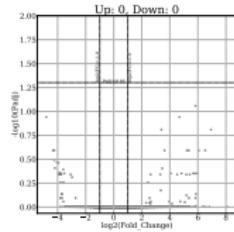
(b) Normal-AIS



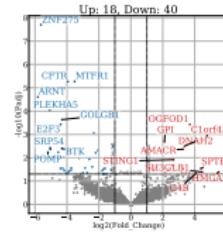
(c) Normal-Primary



(d) AAH-AIS



(e) AAH-Primary

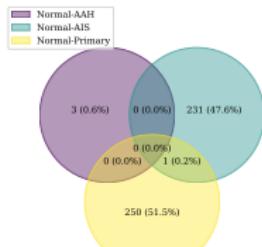


(f) AIS-Primary

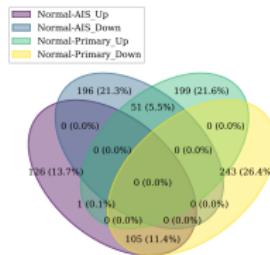
## Figure: DEG Volcano Plots in LUAD

# DEG Venn Diagram in LUAD

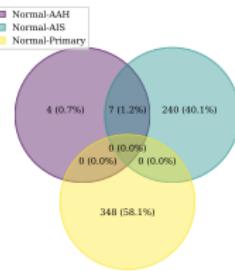
Normal → AAH → AIS → Primary (LUAD)



(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

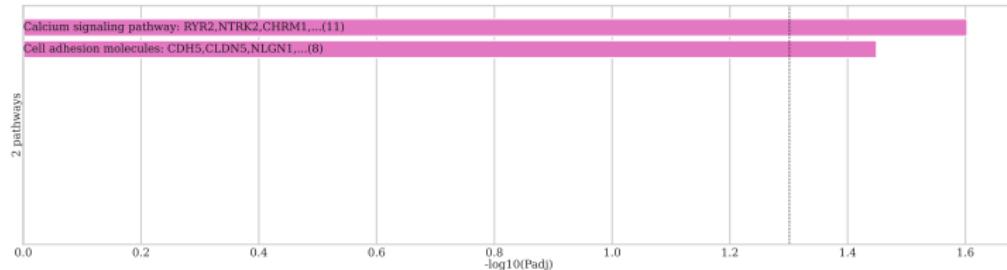


Figure: Up-regulated Pathways on Normal vs. AIS

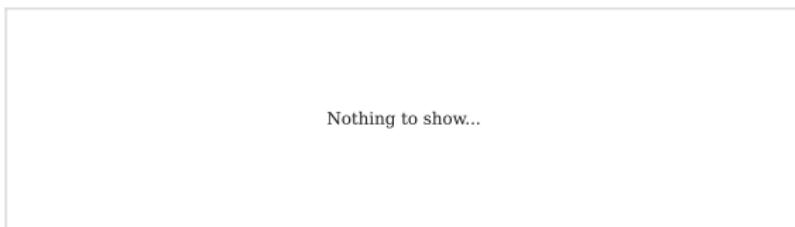


Figure: Down-regulated Pathways on Normal vs. AIS

# Enrichment test with Normal vs. Primary in LUAD



Figure: Up-regulated Pathways on Normal vs. Primary

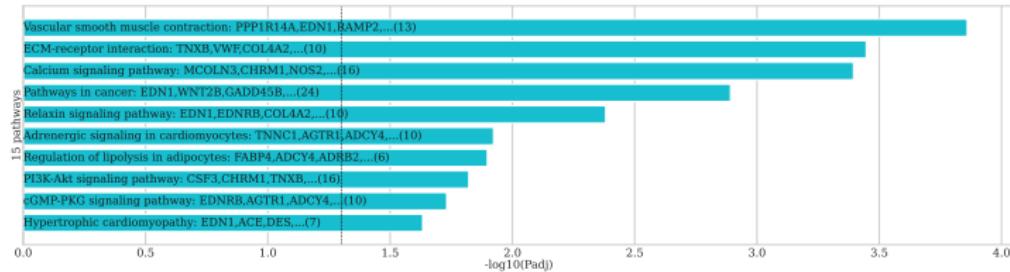


Figure: Down-regulated Pathways on Normal vs. Primary

# Finding in Comparing cancer stage in LUAD I

## ABCA4

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

# 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

### 4.11. Differences in Gene Expression Levels

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

# LUSC Data Composition

Table: Number of WTS LUSC samples

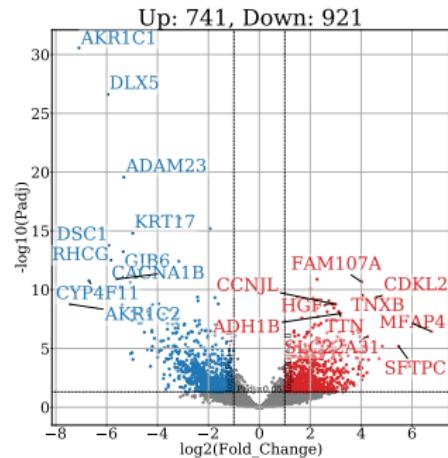
Recurrence?	Stage	Number of Samples	
		Normal	Dysplasia
Recurrence	Normal	1	
	Dysplasia		1
	CIS+AIS	5	
	Primary	6	
	Total	13	
Non-recurrence	Normal	16	
	Dysplasia		1
	CIS+AIS	29	
	Primary	30	
	Total	76	

## Pooled normal samples

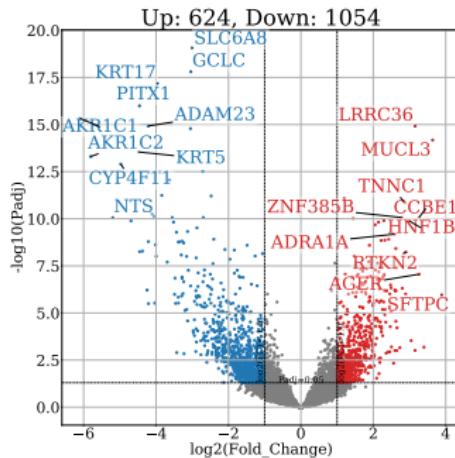
In order to compare with Normal stage, merging Normal samples.

∴ 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

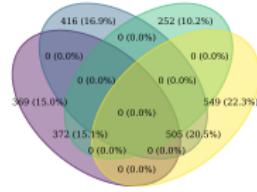
# DEG Venn Diagram for R vs. NR with CIS in LUSC

Recur  
Non-recur



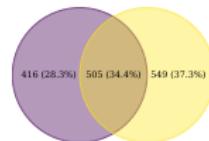
(a) Up-regulated

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

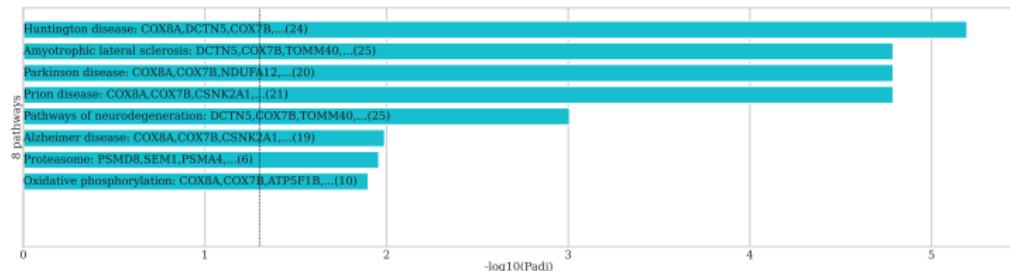


Figure: Down-regulated Pathways for Recur-specific

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

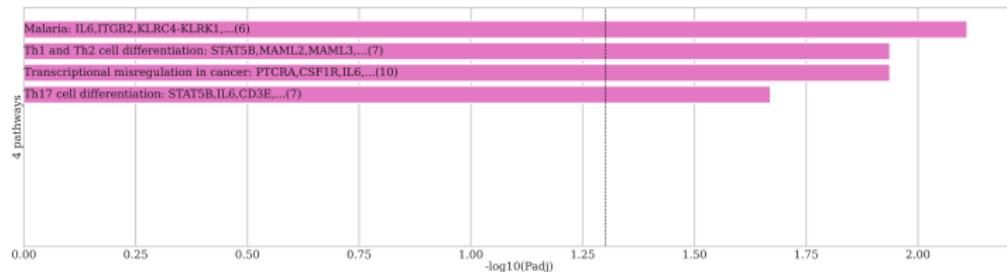


Figure: Up-regulated Pathways for Non-recr-specific

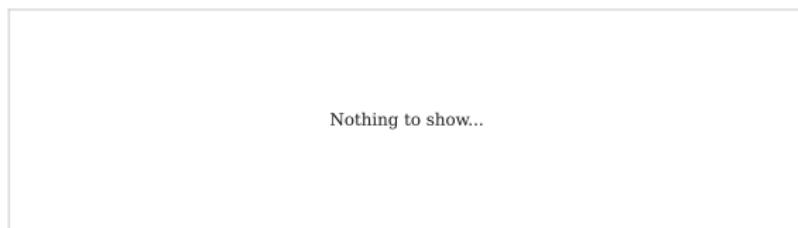


Figure: Down-regulated Pathways for Non-recr-specific

# Enrichment test for Intersected with CIS in LUSC

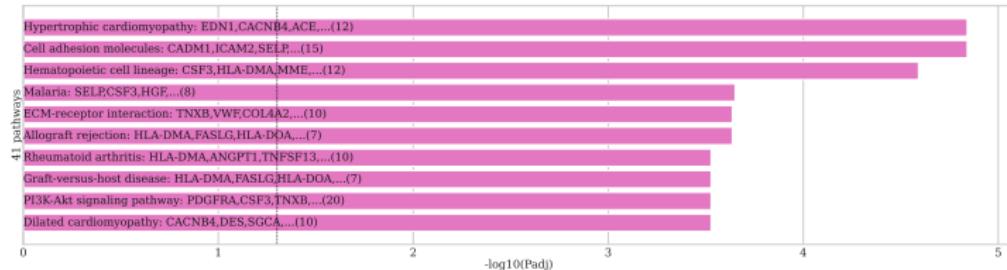


Figure: Up-regulated Pathways for Intersected

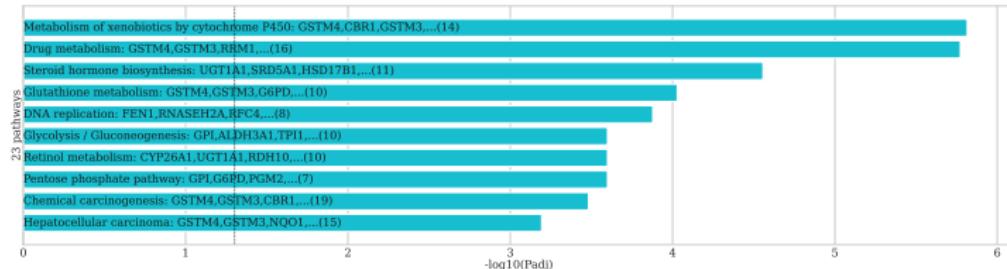
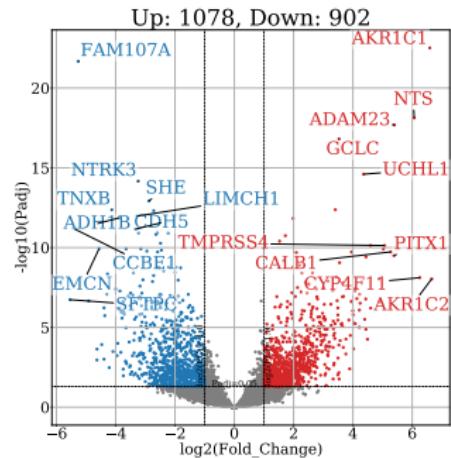
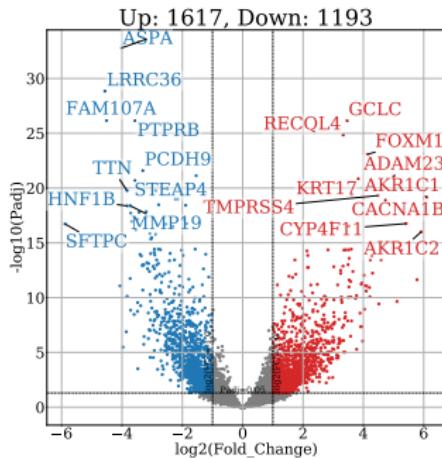


Figure: Down-regulated Pathways for Intersected

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



(a) Recur

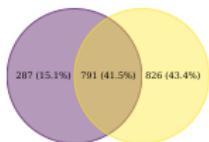


(b) Non-recur

Figure: DEG Volcanot Plot with Primary in LUSC

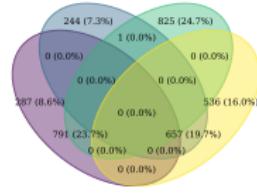
# DEG Venn Diagram for R vs. NR with Primary in LUSC

Recur  
Non-recur



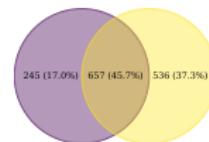
(a) Up-regulated

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

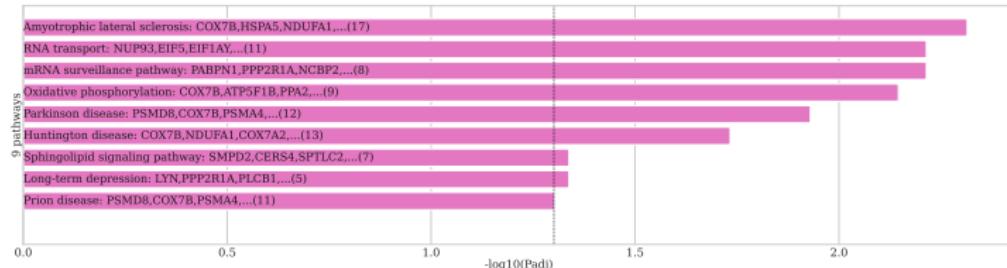


Figure: Up-regulated Pathways for Recur-specific



Figure: Down-regulated Pathways for Recur-specific

# Enrichment test for NR-specific with Primary in LUSC

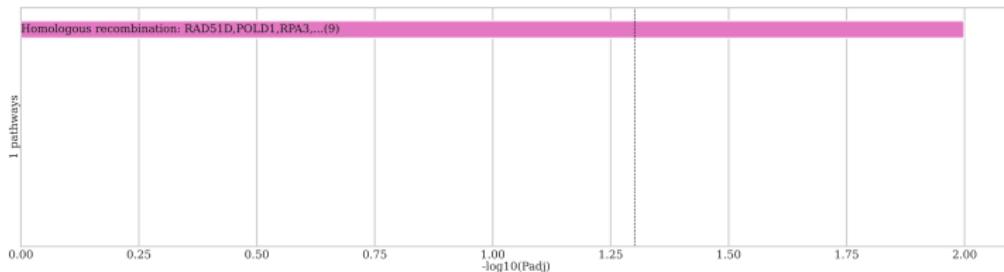


Figure: Up-regulated Pathways for Non-recur-specific

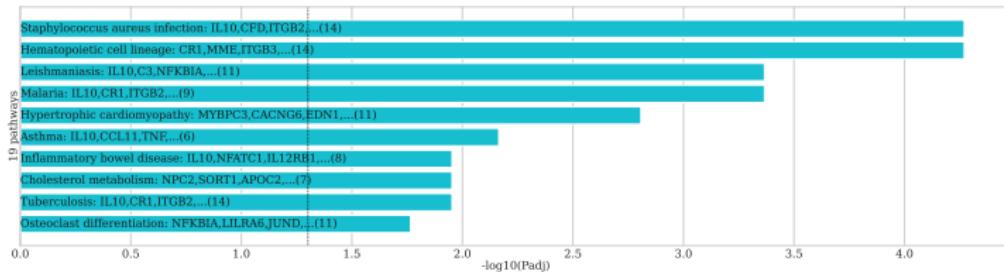


Figure: Down-regulated Pathways for Non-recur-specific

# Enrichment test for Intersected with Primary in LUSC

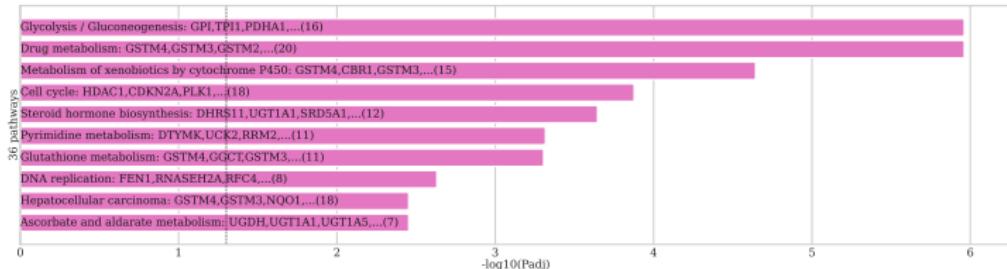


Figure: Up-regulated Pathways for Intersected

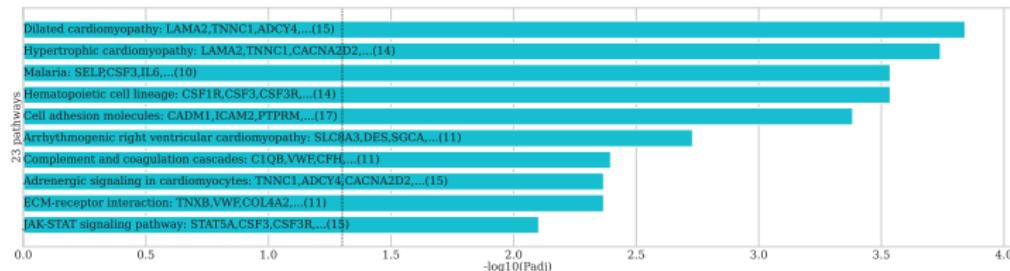


Figure: Down-regulated Pathways for Intersected

# 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

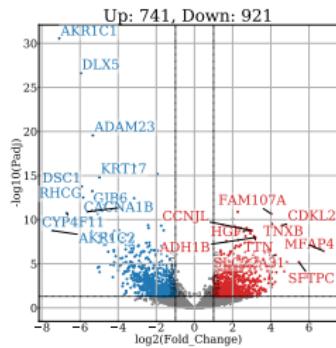
- ① Highly up-regulated in Non-recur patients.
- ② DNA-dependent ATPase (Yin, Kwon, Varshavsky, & Wang, 2004)
- ③ RECQL4 modulate chromosome segregation (Yin et al., 2004)
- ④ RECQL5 promotes metastasis & resistance in NSCLC (Xia, Zhang, Yuan, & Niu, 2021)
- ⑤ RECQL4 ↑ ⇒ Poor prognosis in breast cancer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

## 4. Results

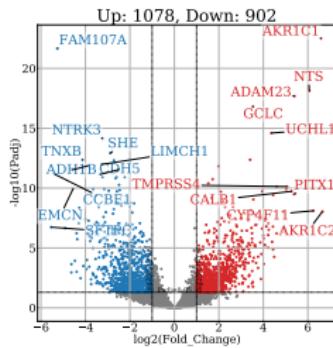
### 4.11. Differences in Gene Expression Levels

#### 4.11.4. Within Recur in LUSC

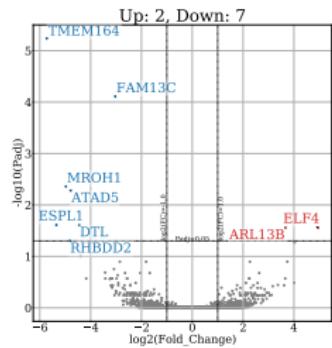
# DEG Volcano Plots with Recur in LUSC



(a) Normal-CIS



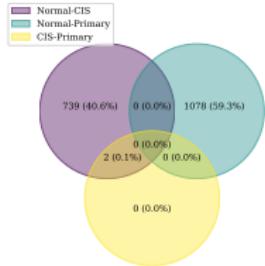
(b) Normal-Primary



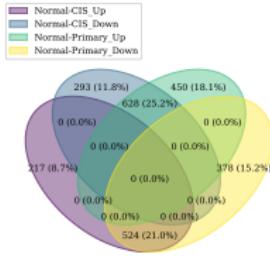
(c) CIS-Primary

Figure: DEG Volcano Plots with Recur samples in LUSC

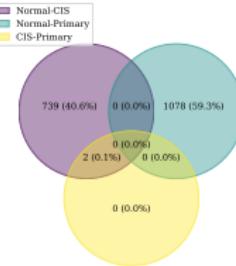
# DEG Venn Diagram with Recur in LUSC



(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

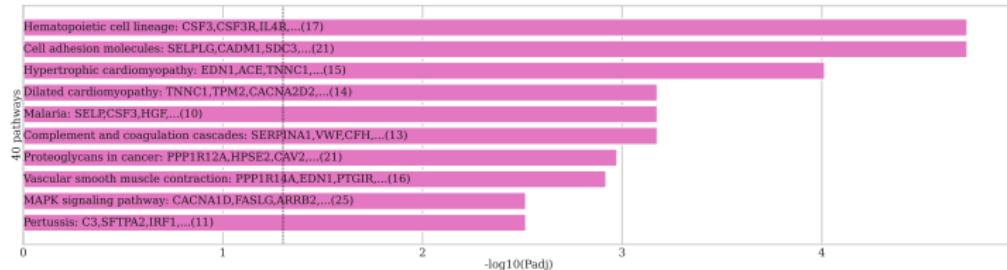


Figure: Up-regulated Pathways on Normal vs. CIS for Recur in LUSC

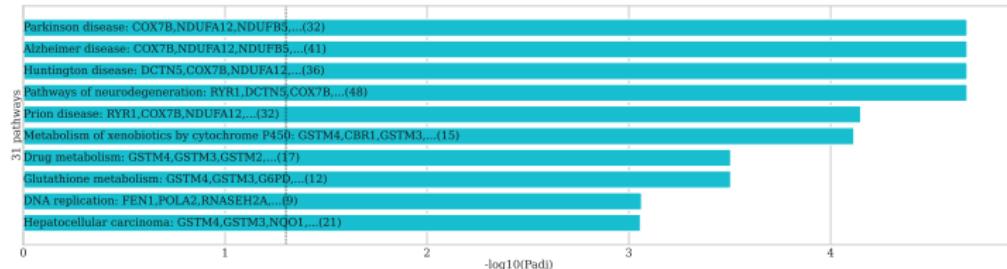


Figure: Down-regulated Pathways on Normal vs. CIS for Recur in LUSC

# Enrichment test with Normal vs. Primary for Recur

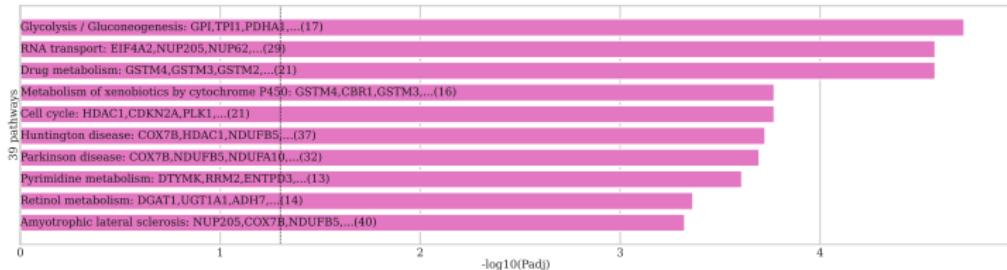


Figure: Up-regulated Pathways on Normal vs. Primary for Recur in LUSC

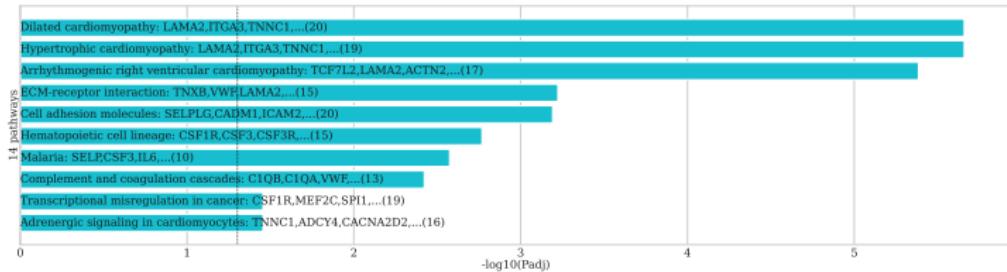


Figure: Down-regulated Pathways on Normal vs. Primary for Recur in LUSC

# 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

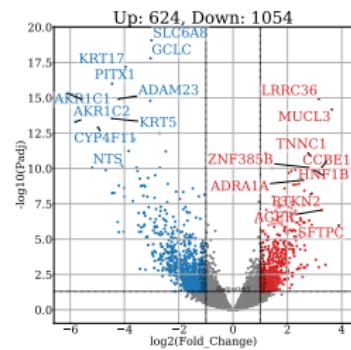
- ① Up-regulated in CIS, but down-regulated in Primary.
- ② May play a role in tumor development (L. Wang et al., 2000)
- ③ Negatively regulates focal adhesion assembly (Le et al., 2010)

## 4. Results

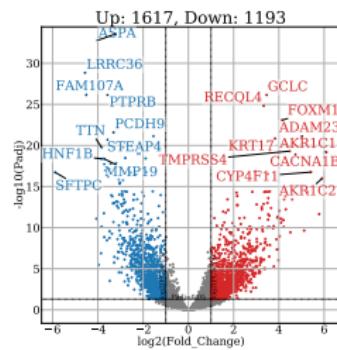
### 4.11. Differences in Gene Expression Levels

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

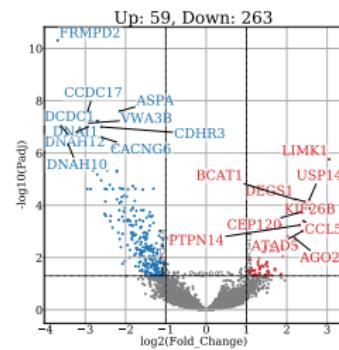
## DEG Volcano Plots with Non-recur in LUSC



(a) Normal-CIS



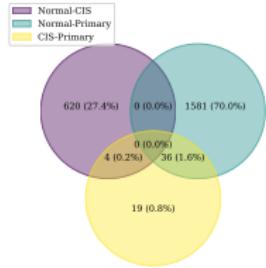
(b) Normal-Primary



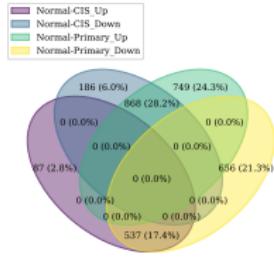
(c) CIS-Primary

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

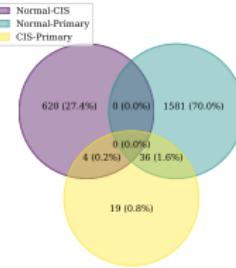
# DEG Venn Diagram with Non-recur in LUSC



(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

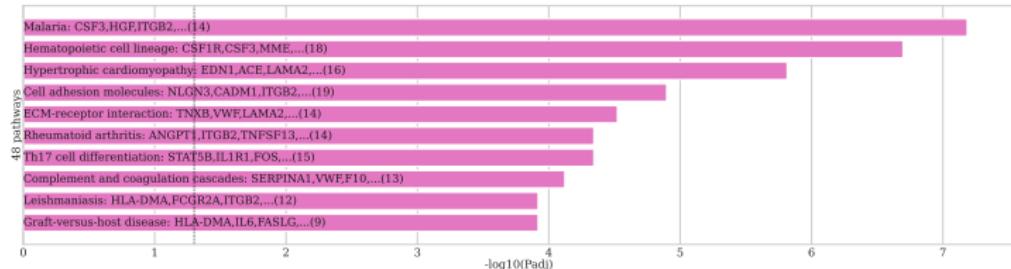


Figure: Up-regulated Pathways on Normal vs. CIS for Non-recur in LUSC

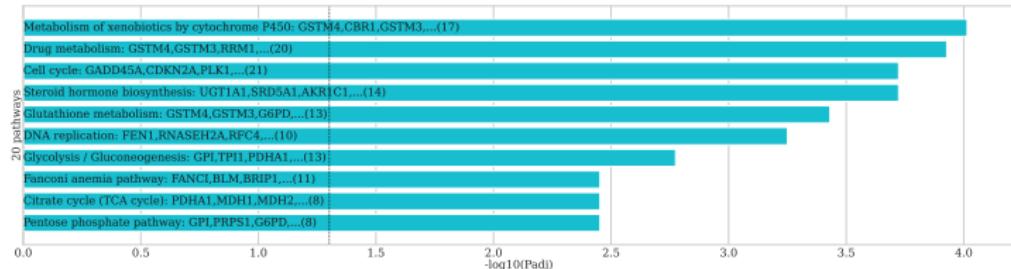


Figure: Down-regulated Pathways on Normal vs. CIS for Non-recur in LUSC

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

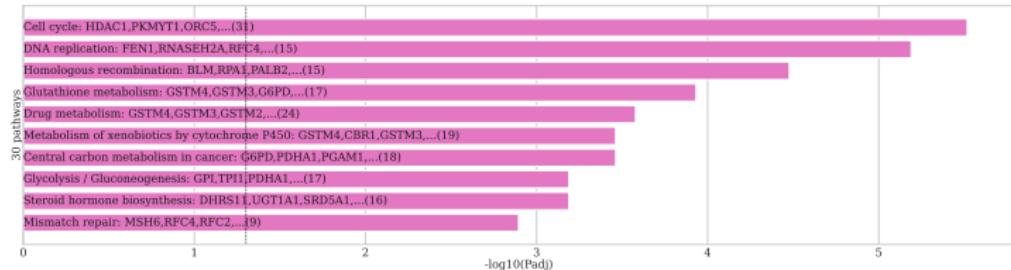


Figure: Up-regulated Pathways on Normal vs. Primary for Non-recur in LUSC

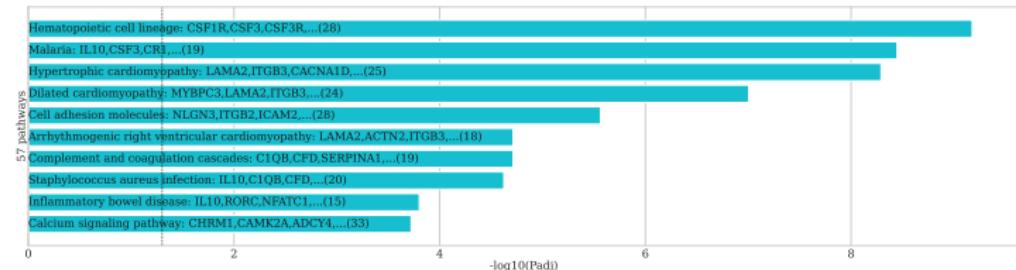


Figure: Down-regulated Pathways on Normal vs. Primary for Non-recur in LUSC

# 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

### 4.11. Differences in Gene Expression Levels

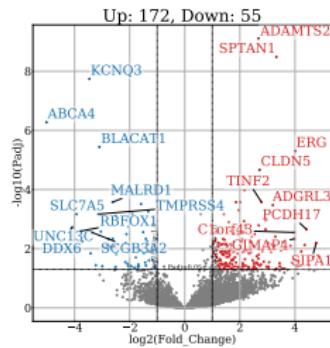
#### 4.11.6. Within Non-recur in LUAD

# LUAD Data Composition

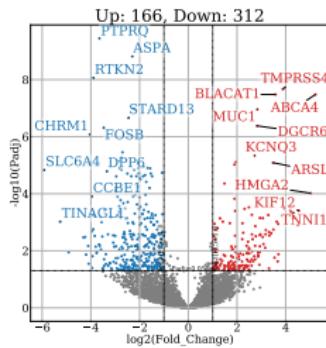
Table: Number of WTS LUAD samples

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

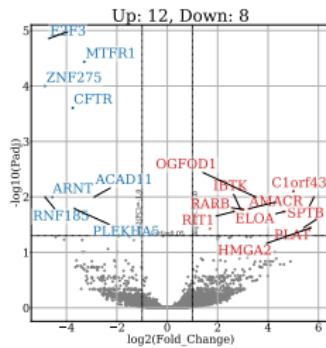
# DEG Volcano Plots with Non-recr in LUAD



(a) Normal-AIS



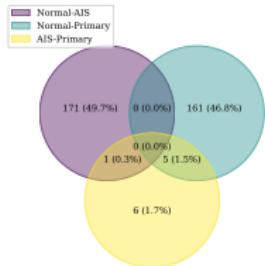
(b) Normal-Primary



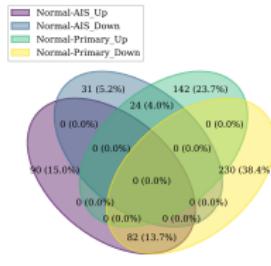
(c) AIS-Primary

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

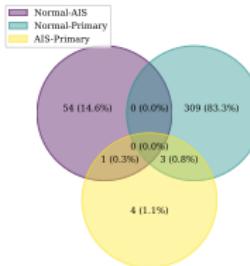
# DEG Venn Diagram with Non-recur in LUAD



(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

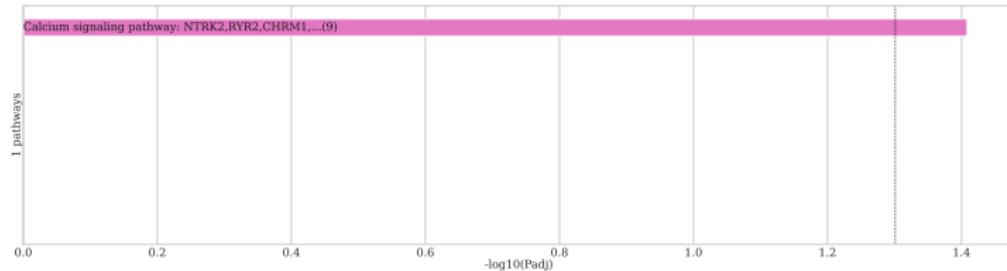


Figure: Up-regulated Pathways on Normal vs. AIS for Non-recur in LUAD

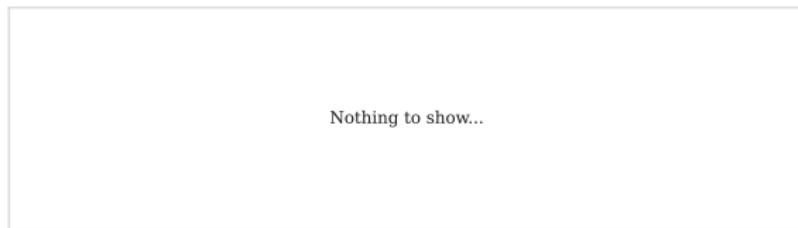


Figure: Down-regulated Pathways on Normal vs. AIS for Non-recur in LUAD

# Enrichment test with Normal vs. Primary in LUAD



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

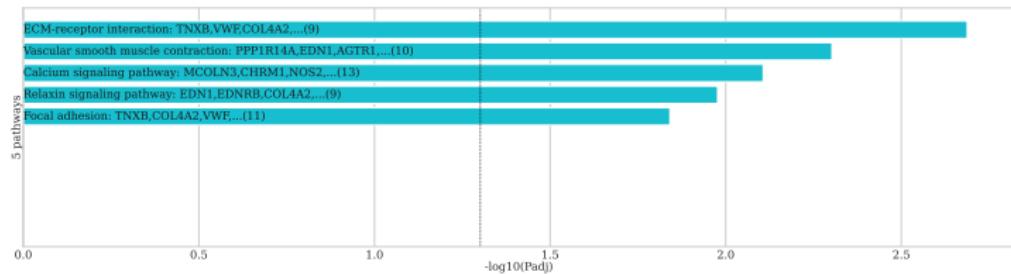


Figure: Down-regulated Pathways on Normal vs. Primary for Non-recur in LUAD

## 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

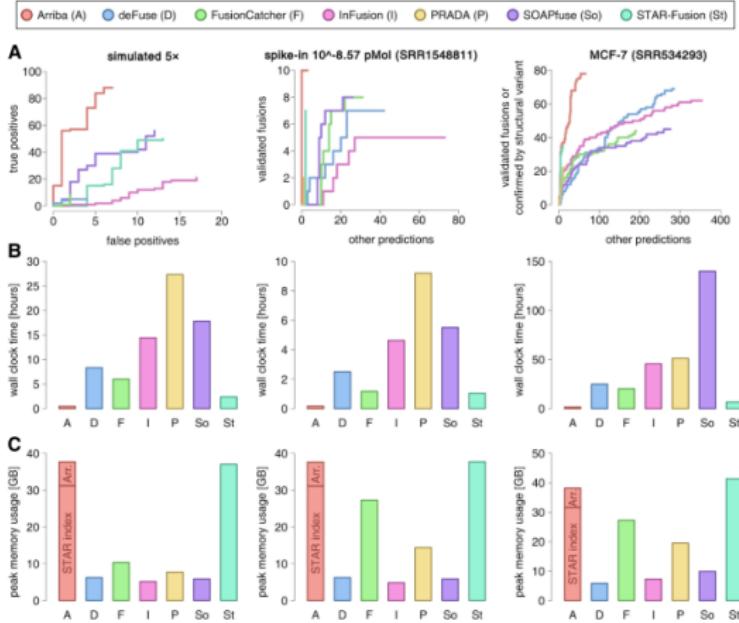
- ① Down-regulated in AIS, but up-regulated in Primary.
- ② Bladder cancer-associated transcript 1
- ③ Chemo-resistance of NSCLC (Huang et al., 2019)
- ④ Predicts poor prognosis in SCLC (W. Chen et al., 2019)
- ⑤ Up-regulated in many human cancers (Ye, Yang, Liu, Lv, & Ye, 2020)

# Findings in DEG Analysis

## 4. Results

### 4.12. 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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