

Lung Precancer Analysis

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Overview

1 Introduction

2 Materials

3 Methods

4 Results

5 Discussion

6 References

1. Introduction

1. Introduction

1.1. Lung Cancer

Lung Cancer?

The most common cancer

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

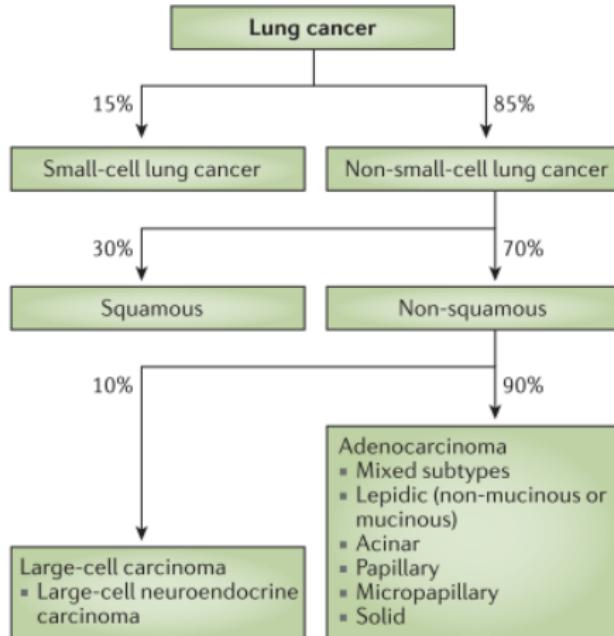


Figure: Lung cancer classification (Gridelli et al., 2015)

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

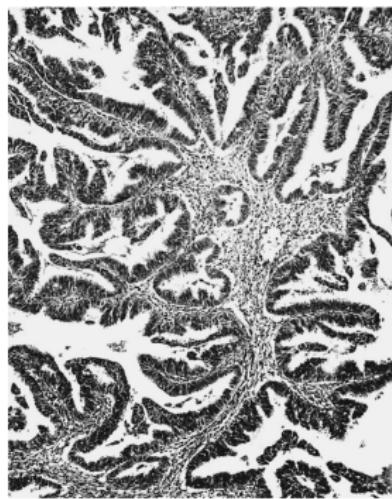
TCGA LUSC (Dosenbach et al., 2007)

- 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

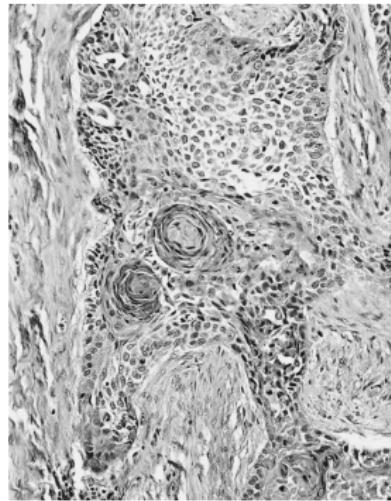
1. Introduction

1.5. LUAD vs. LUSC

LUAD vs. LUSC I



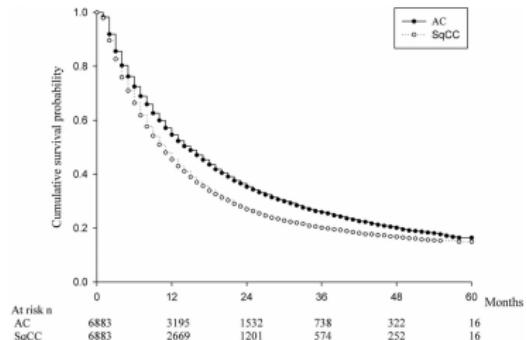
(a) LUAD



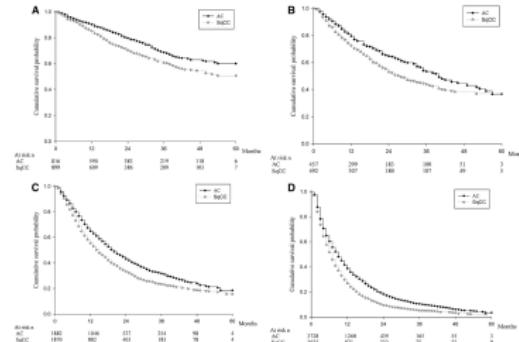
(b) LUSC

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 $n=18$)
 - ② Normal → Dysplasia → CIS → LUSC (patient $n=77$)

2. Materials

2.1. WES Data

WES Data Composition

Table: Number of WES samples

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

WES Data Composition with Recurrence I

Table: LUSC WES Data with Recurrence

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

WES Data Composition with Recurrence II

Table: LUAD WES Data with Recurrence

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

WES Data Composition with Smoking I

Table: LUSC WES Data with Smoking

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

WES Data Composition with Smoking II

Table: LUAD WES Data with Smoking

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

2. Materials

2.2. WTS Data

WTS Data Composition

Table: Number of WTS samples

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

WTS Data Composition with Recurrence I

Table: LUSC WTS Data with Recurrence

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

WTS Data Composition with Recurrence II

Table: LUAD WTS Data with Recurrence

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

WTS Data Composition with Smoking I

Table: LUSC WTS Data with Smoking

Smoking?	Stage	Number of Samples	
		Normal	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

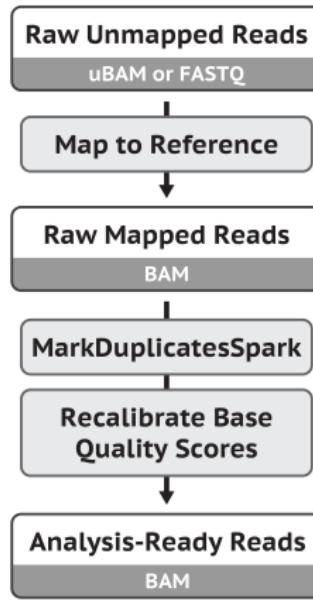


Figure: Data pre-processing for variant discovery (Van der Auwera et al., 2013; DePristo et al., 2011)

Somatic short variant discovery

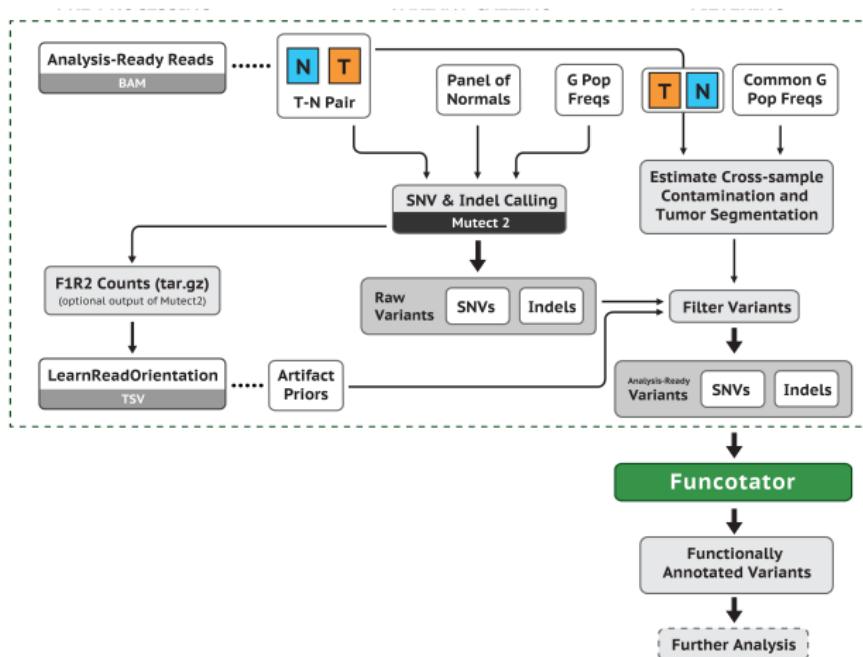


Figure: Somatic short variant (SNVs + Indels) discovery workflow (Van der Auwera et al., 2013; DePristo et al., 2011)

Germline short variant discovery

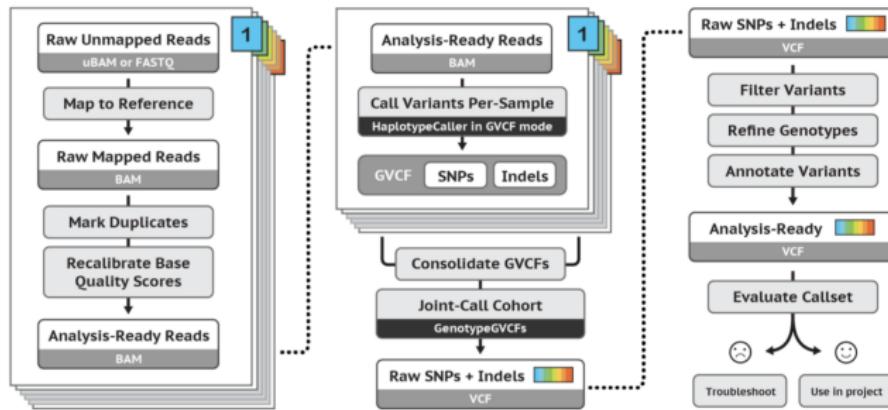


Figure: Germline short variant (SNVs + Indels) discovery workflow (Van der Auwera et al., 2013; DePristo et al., 2011)

RNA-seq short variant discovery

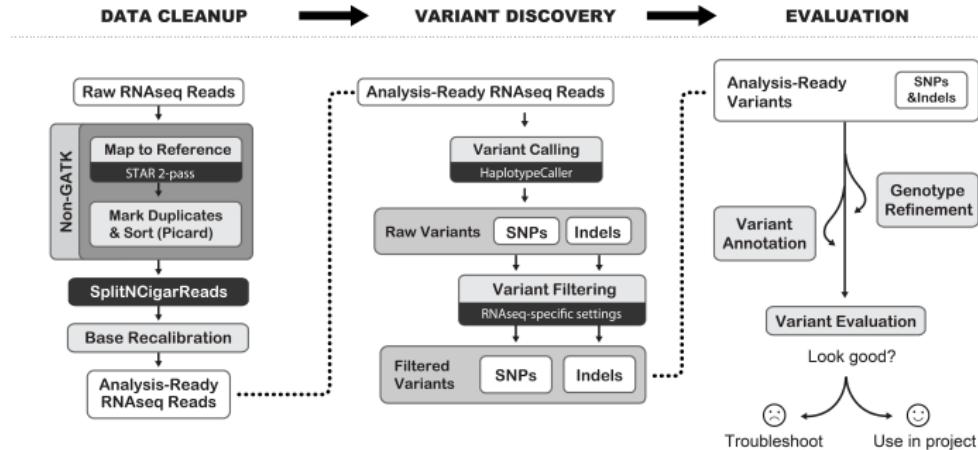


Figure: RNA-seq short variant (SNVs + Indels) discovery workflow (Van der Auwera et al., 2013; DePristo et al., 2011)

4. Results

4. Results

4.1. Quality Checks with FastQC

FastQC?

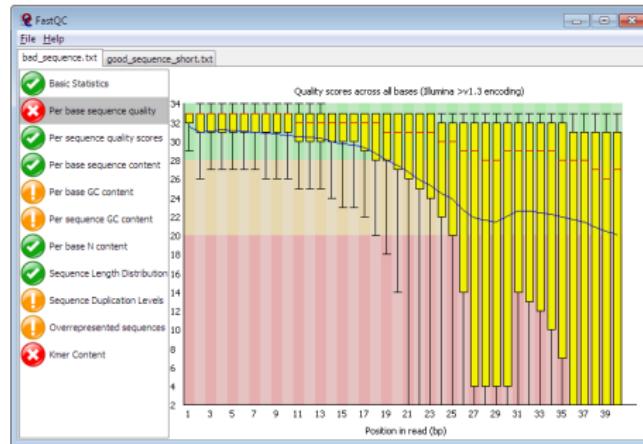


Figure: Example of FastQC Result (Andrews et al., 2012)

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

FastQC on WES

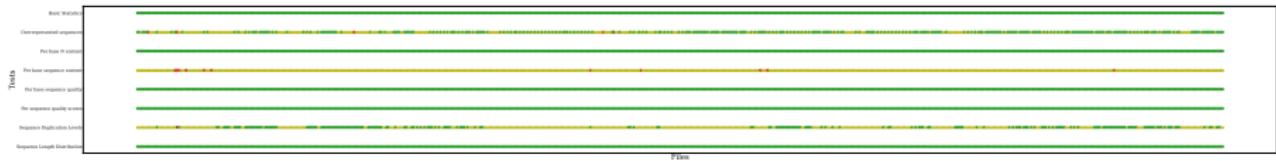
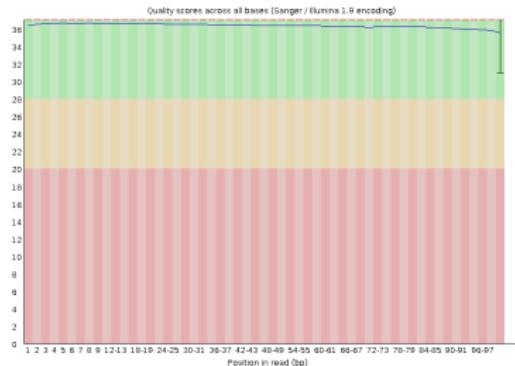


Figure: FastQC with WES data

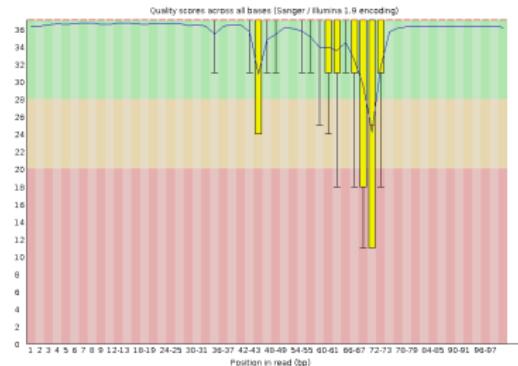
Failure on 33P1 sample

33P1 is excluded from further analyses.

Failure on 33P1 I



(a) 33N



(b) 33P1

Figure: Per Base Sequence Quality Results

Failure on 33P1 II

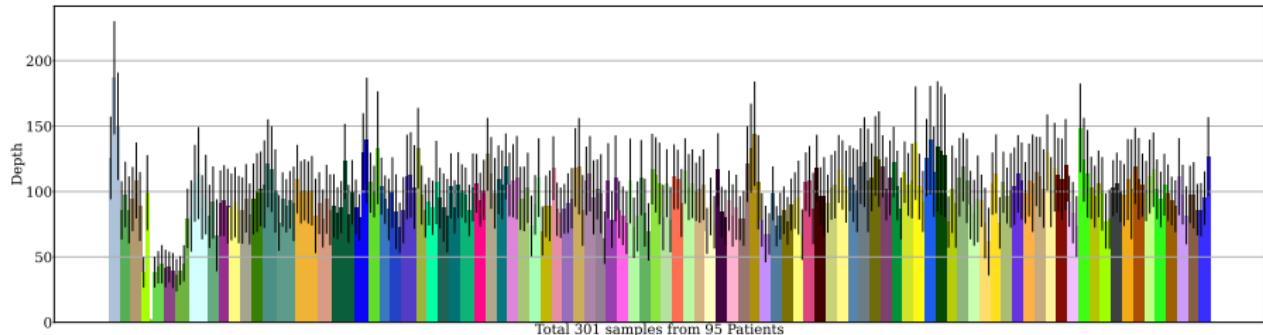


Figure: Coverage Depth Plot

- 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

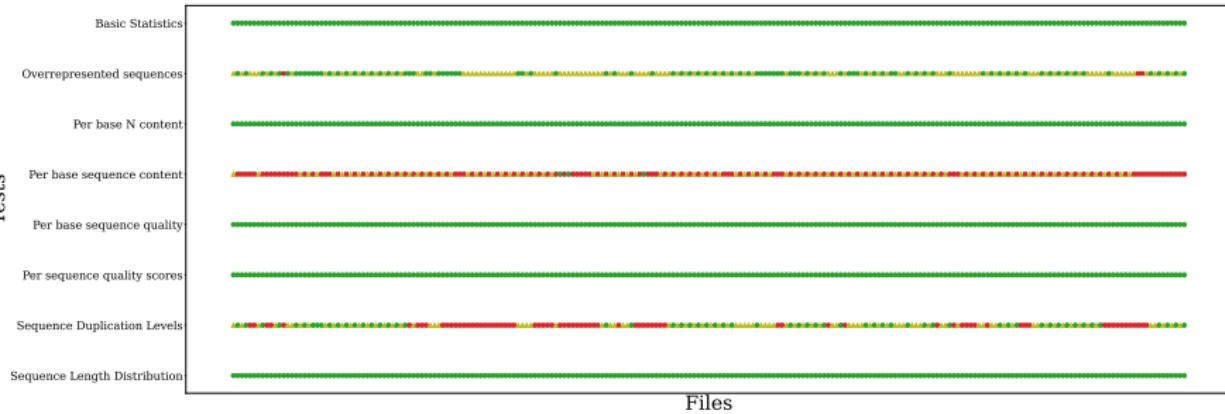


Figure: FastQC with WTS data

All sample are good to analysis

∴ No sample has more than 5 failures.

4. Results

4.2. Quality Checks with Picard

Picard?

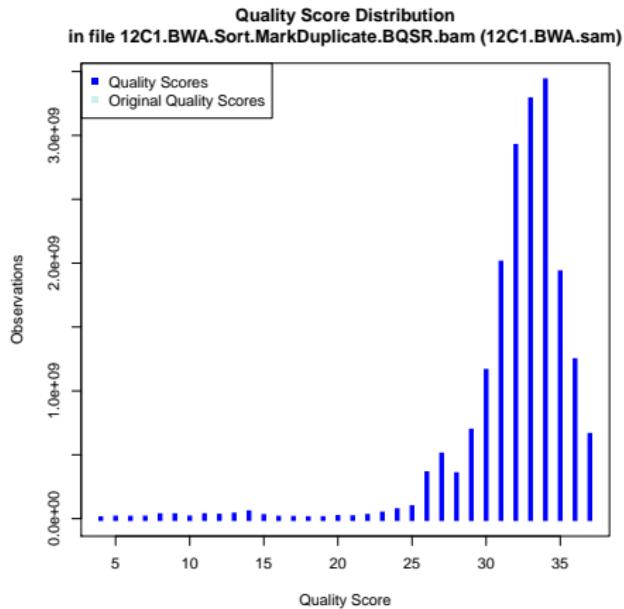


Figure: Quality Distribution of 12C1 sample

Quality Distribution Plot

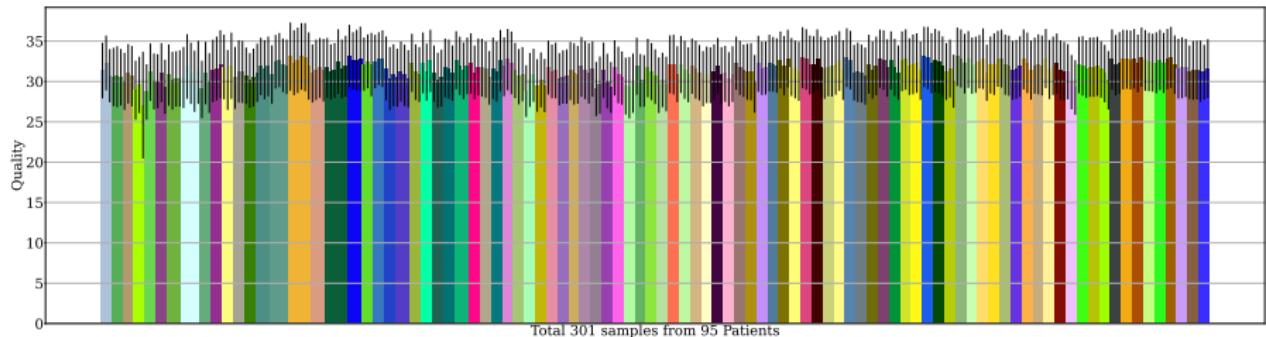


Figure: Quality Distribution by Samples

Findings in Picard

4. Results

4.3. Copy Number Variation Analysis with PureCN

PureCN?

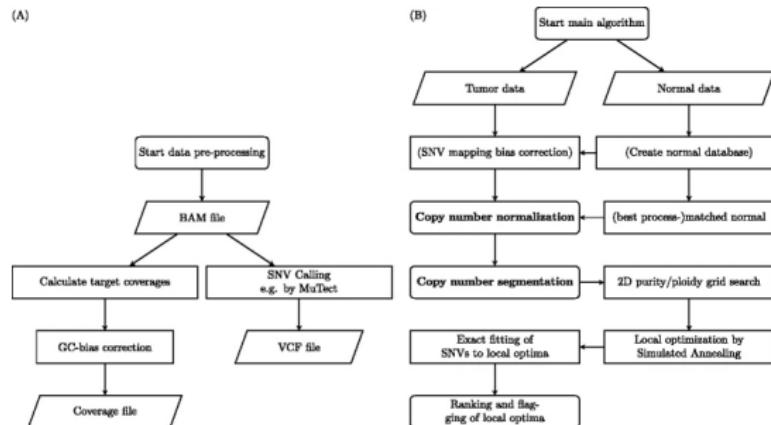
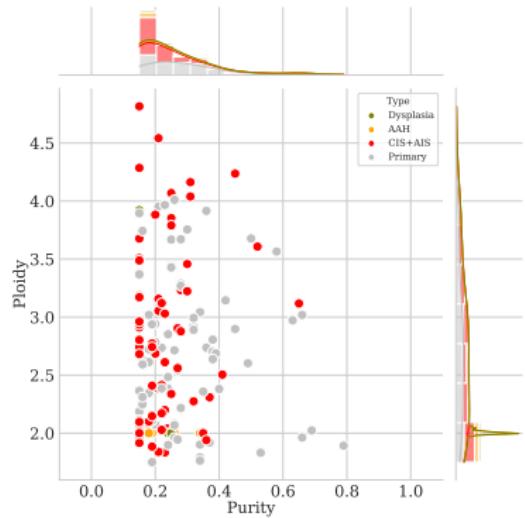
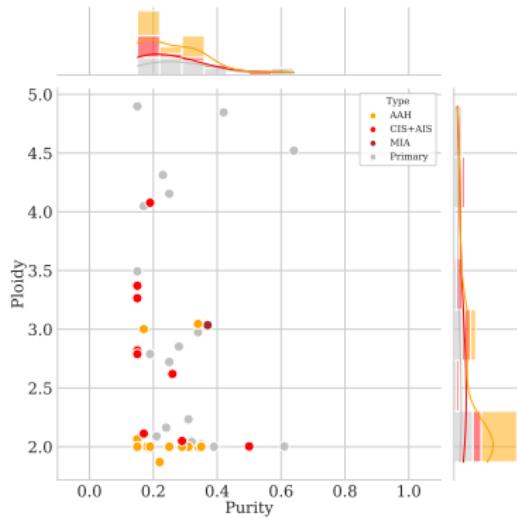


Figure: Flowchart of the PureCN data pre-processing pipeline (Riester et al., 2016)

Purity & Ploidy on WES



(a) LUSC Samples



(b) LUAD Samples

Figure: Cellularity and Ploidy from PureCN

LUSC in CNV Plot I

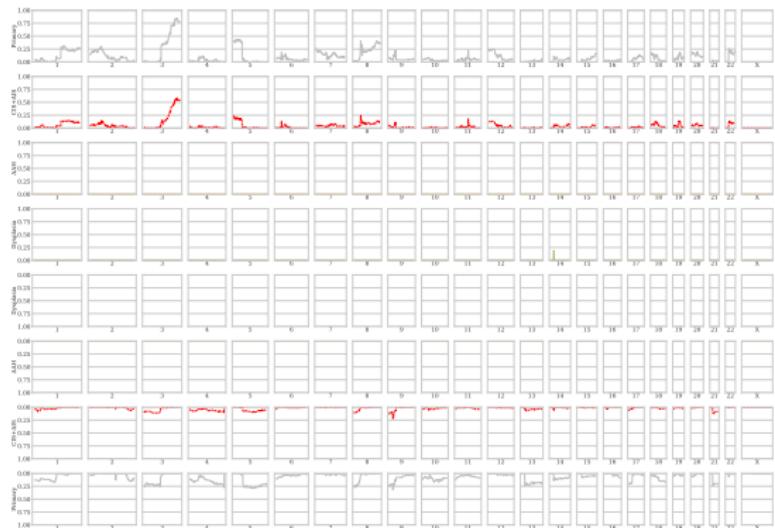


Figure: LUSC in CNV Plot

LUSC in CNV Plot II

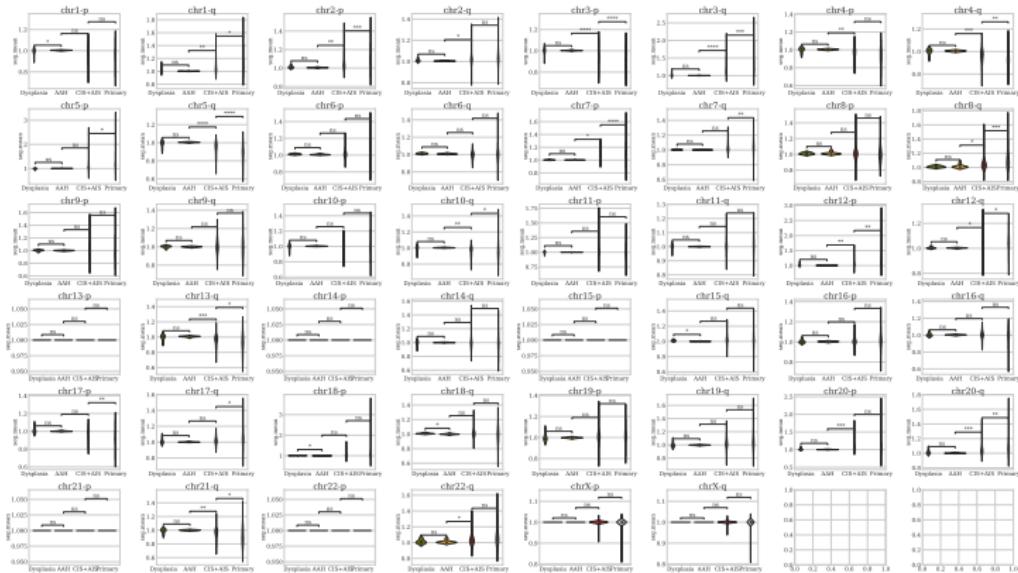


Figure: LUSC in Violin Plots

LUSC with Recurrence in CNV Plot I



Figure: LUSC with Recurrence in CNV Plot

LUSC with Recurrence in CNV Plot II

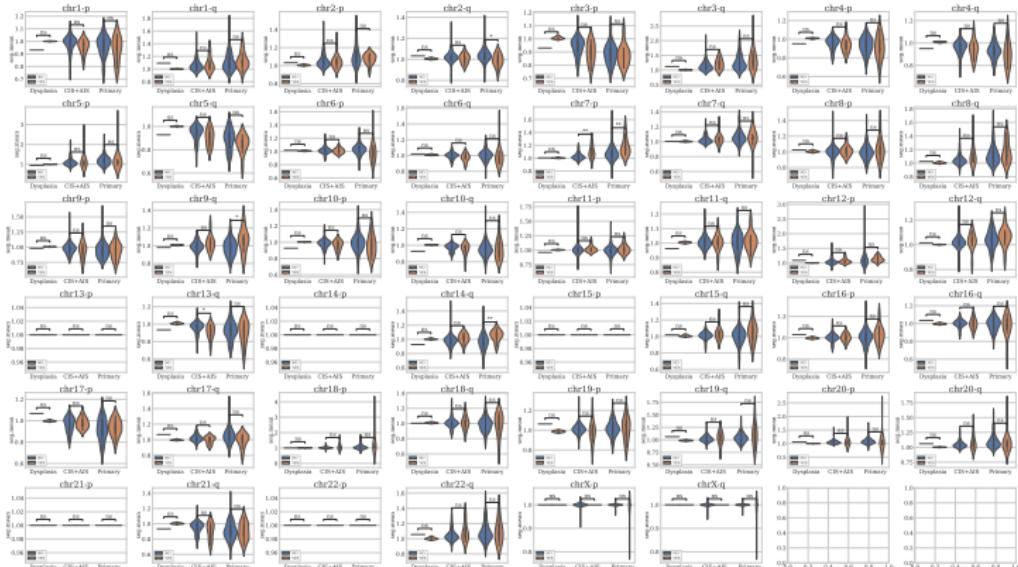


Figure: LUSC with Recurrence in Violin Plots

LUSC with Smoking in CNV Plot

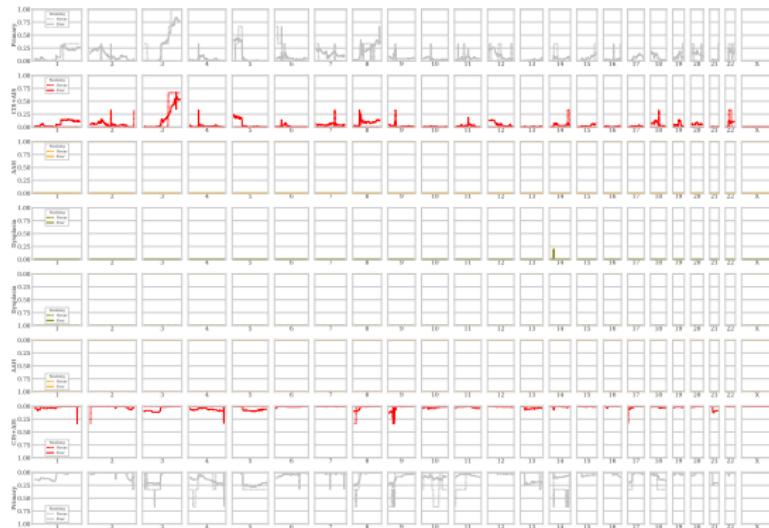


Figure: LUSC with Smoking in CNV Plot

LUSC with Smoking in CNV Plot II

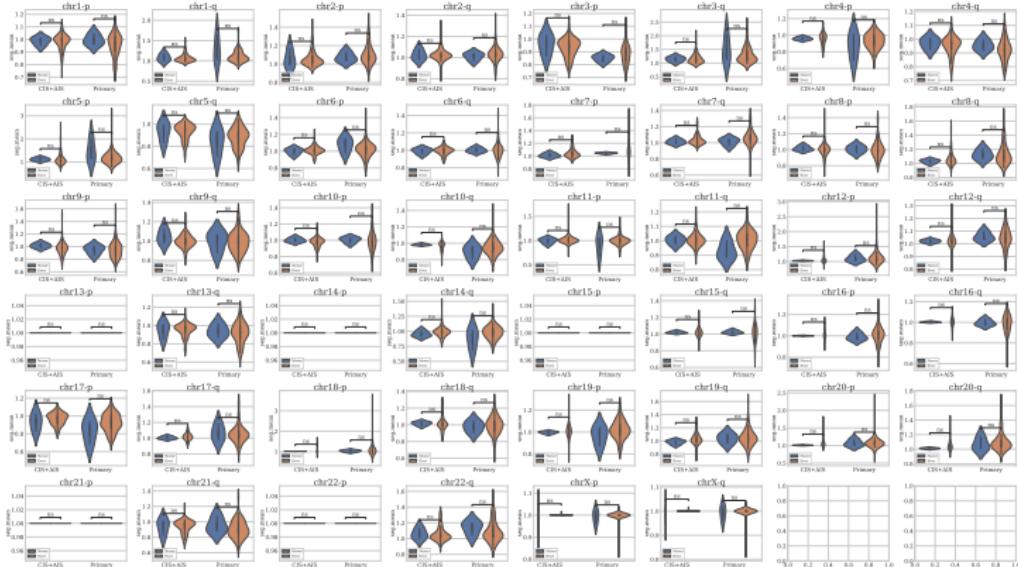


Figure: LUSC with Smoking in Violin Plots

Findings in PureCN with LUSC I

Chr2 p-arm gain

- Chr2 p-arm gain in Primary.

Table: CGC Tier1 genes in Chr2 p-arm

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

Findings in PureCN with LUSC II

Chr2 q-arm loss

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

Table: CGC Tier1 genes in Chr2 q-arm

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

Findings in PureCN with LUSC III

Chr3 p-arm loss

- ① Chr3 p-arm loss in Primary.

Table: CGC Tier1 genes in Chr3 p-arm

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

Findings in PureCN with LUSC IV

Chr3 q-arm gain

- ① Chr3 q-arm gain in Primary.

Table: CGC Tier1 genes in Chr3 q-arm

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

Findings in PureCN with LUSC V

Chr5 q-arm loss

① Chr5 q-arm loss in Primary.

Table: CGC Tier1 genes in Chr5 q-arm

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

Findings in PureCN with LUSC VI

Chr7 p-arm gain

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

Table: CGC Tier1 genes in Chr7 p-arm

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

Findings in PureCN with LUSC VII

Chr8 q-arm gain

① Chr8 q-arm gain in Primary.

Table: CGC Tier1 genes in Chr8 q-arm

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

Findings in PureCN with LUSC VIII

Chr9 q-arm gain

- Chr9 q-arm gain in Recurrence & Primary.

Table: CGC Tier1 genes in Chr9 q-arm

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

Findings in PureCN with LUSC IX

Chr14 q-arm gain

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

Table: CGC Tier1 genes in Chr14 q-arm

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

Findings in PureCN with LUSC X

Chr19 p-arm gain

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

Table: CGC Tier1 genes in Chr19 p-arm

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

LUAD in CNV Plot I

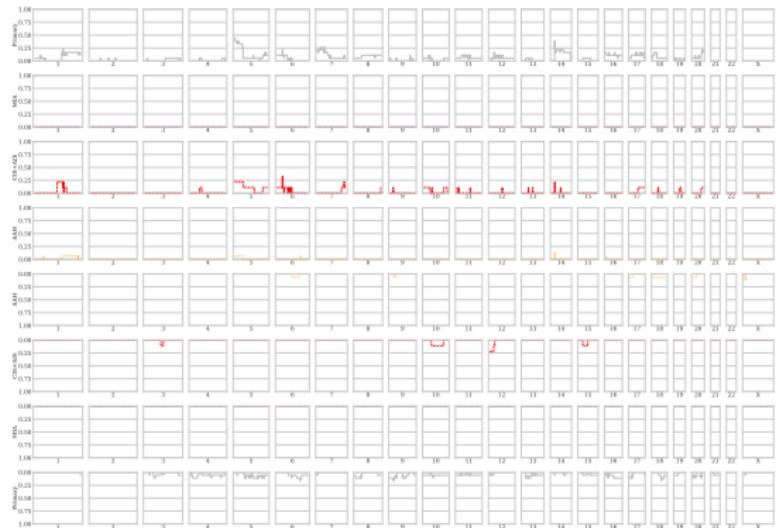


Figure: LUAD in CNV Plot

LUAD in CNV Plot II

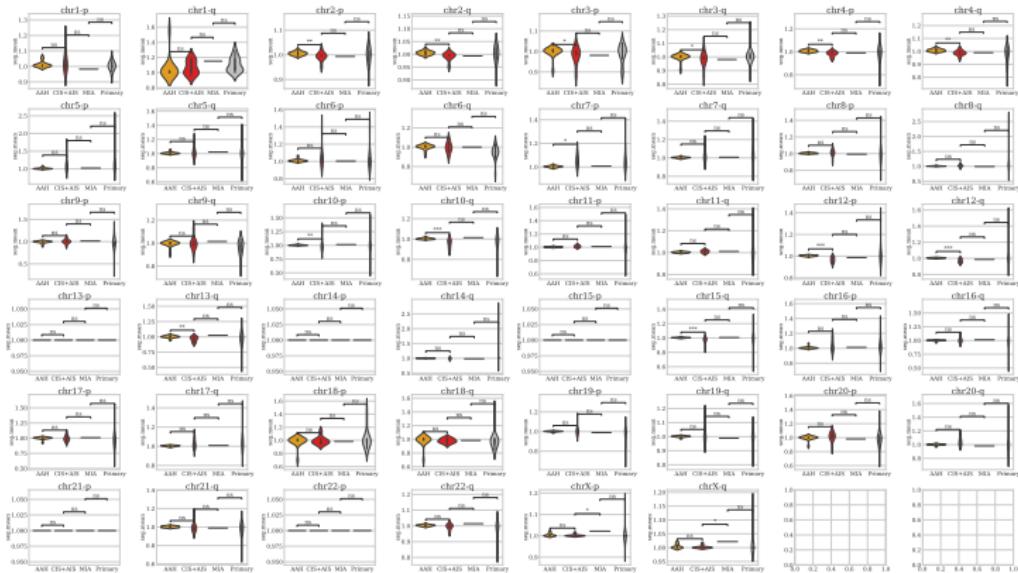


Figure: LUAD in Violin Plots

LUAD with Recurrence in CNV Plot

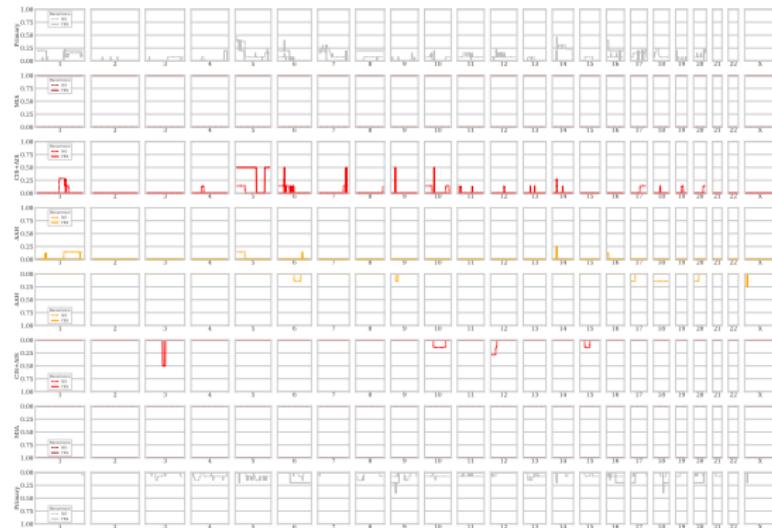


Figure: LUAD with Recurrence in CNV Plot

LUAD with Recurrence in CNV Plot II



Figure: LUAD with Recurrence in Violin Plots

LUAD with Smoking in CNV Plot I



Figure: LUAD with Smoking in CNV Plot

LUAD with Smoking in CNV Plot II

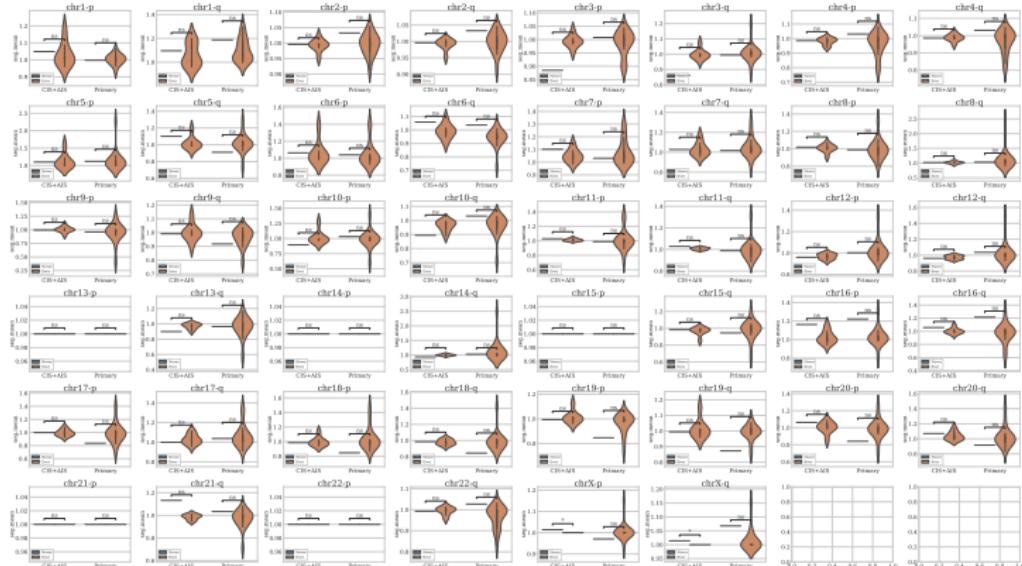


Figure: LUAD with Smoking in Violin Plots

Findings in PureCN with LUAD I

Chr1 q-arm gain

- ① Chr1 q-arm gain in Primary.

Table: CGC Tier1 genes in Chr1 q-arm

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

Findings in PureCN with LUAD II

Chr4 q-arm gain

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

Table: CGC Tier1 genes in Chr4 q-arm

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

Findings in PureCN with LUAD III

Chr5 q-arm gain

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

Table: CGC Tier1 genes in Chr5 q-arm

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

Findings in PureCN with LUAD IV

Chr6 p-arm gain

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

Table: CGC Tier1 genes in Chr6 p-arm

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

Chr6 q-arm gain

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

Table: CGC Tier1 genes in Chr6 q-arm

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

Findings in PureCN with LUAD VI

Chr6 q-arm loss

- Chr6 q-arm loss in Primary.

Table: CGC Tier1 genes in Chr6 q-arm

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

Findings in PureCN with LUAD VII

Chr7 q-arm gain

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

Table: CGC Tier1 genes in Chr7 q-arm

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

Findings in PureCN with LUAD VIII

Chr12 p-arm gain

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

Table: CGC Tier1 genes in Chr12 p-arm

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

Findings in PureCN with LUAD IX

Chr12 q-arm gain

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

Table: CGC Tier1 genes in Chr12 q-arm

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

Findings in PureCN with LUAD X

Chr22 q-arm loss

- ① Chr22 q-arm loss in Primary.

Table: CGC Tier1 genes in Chr22 q-arm

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

Findings in PureCN

4. Results

4.4. Copy Number Variation Analysis with Gistic

Gistic?

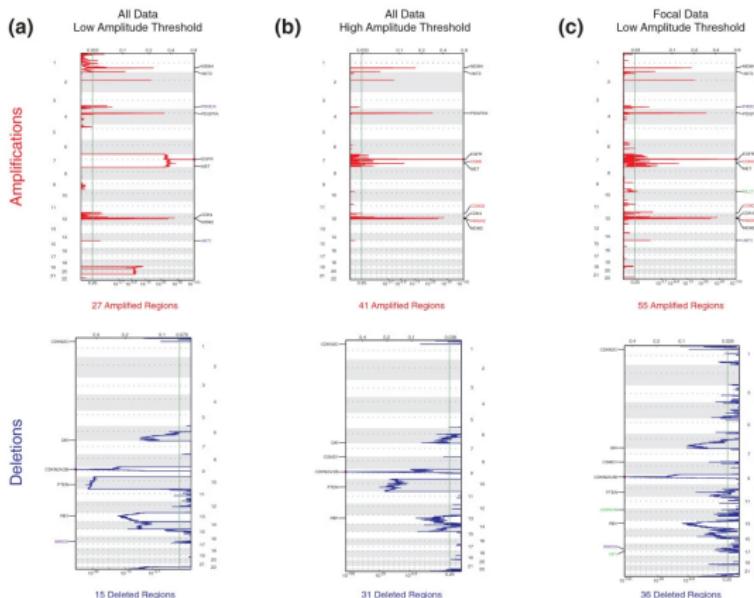


Figure: Effects of arm-level events on GISTIC results (Mermel et al., 2011)

4. Results

4.4. Copy Number Variation Analysis with Gistic

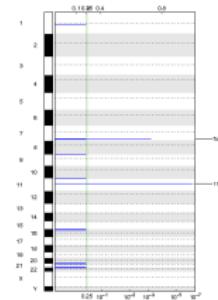
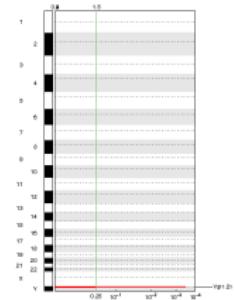
4.4.1. Gistic in LUSC

LUSC Data Composition

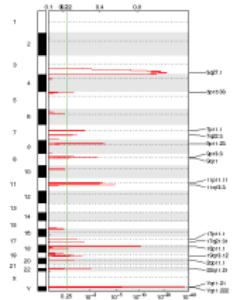
Table: Number of WES samples

Cancer Subtype	Stage	Number of Samples	
		Normal	77
LUSC	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

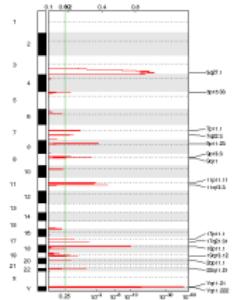
Gistic in LUSC



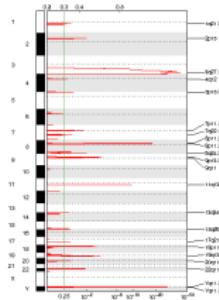
(a) Dysplasia



(b) CIS



(c) Precancer

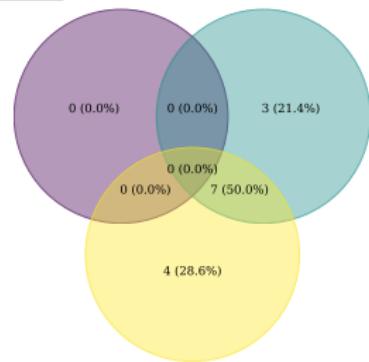


(d) Primary

Figure: Gistic results in LUSC

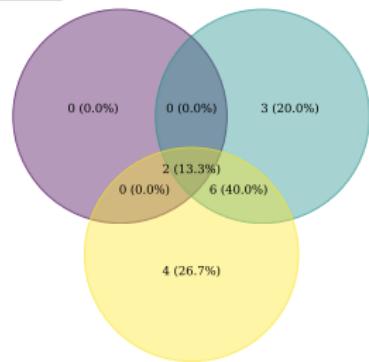
Peaks in LUSC I

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

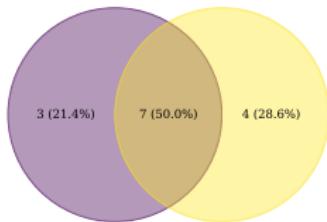
Peaks	Dysplasia	CIS	Primary	CGC Genes
1q21.3			*	ARNT,MLLT11,S100A7,SETDB1,TPM3
2p15			*	XPO1
3q27.1	*	*		
4q12			*	CHIC2,FIP1L1,KDR,KIT,PDGFRA
5p15.33	*	*		SDHA,TERT
7p11.1	*	*		
7q22.1			*	CUX1,TRRAP
7q22.3	*			
8p11.21			*	ANK1,HOOK3,IKBKB,KAT6A
8p11.23	*	*		FGFR1,NSD3
8q24.3			*	RECQL4
9p13.3	*	*		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

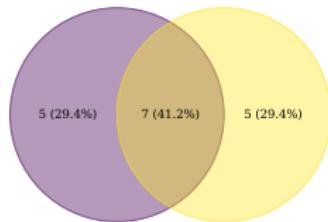
Table: Deletion Peaks in LUSC – stage

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

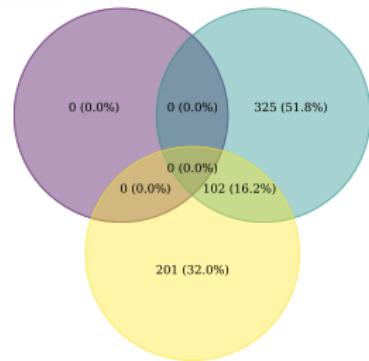
Peaks in LUSC VI

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	*		

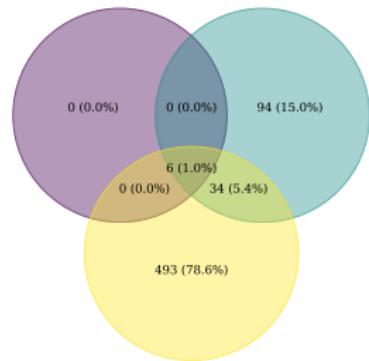
Genes in LUSC I

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

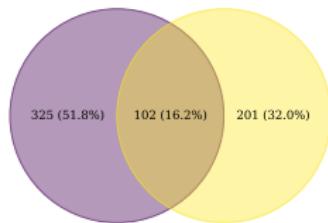
CGC Genes	Dysplasia	CIS	Primary
AKT2	*		
ANK1		*	
BCL11A		*	
CCND1	*		
CEBPA	*		
CEP89	*		
DGCR8	*	*	
FANCG	*		
FGFR1	*	*	
HOOK3		*	
IKBKB		*	
KAT6A		*	
LSM14A	*		
LZTR1	*	*	
PAX5	*		
PIK3CA	*		
RECQL4		*	
REL		*	
SOX2	*		
XPO1		*	

Genes in LUSC III

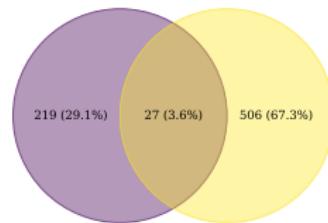
Table: Deletion Genes in LUSC – stage

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

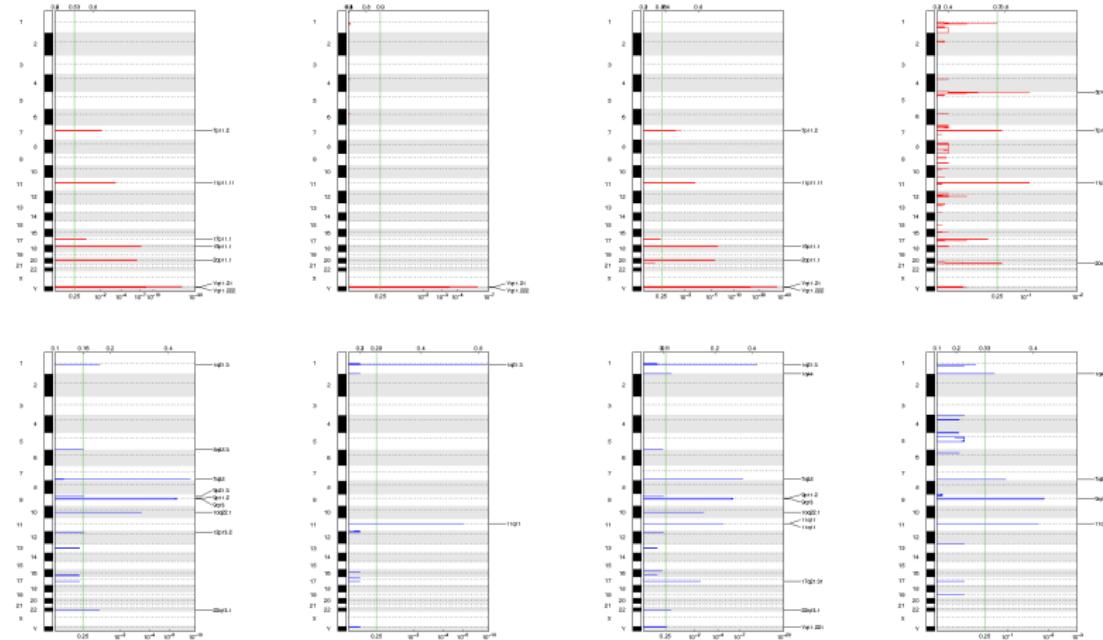
4.4.2. Gistic in LUAD

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

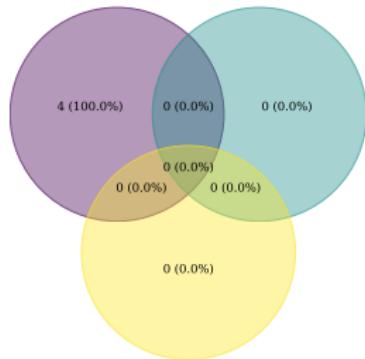
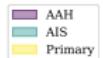
(b) AIS

(c) Precancer

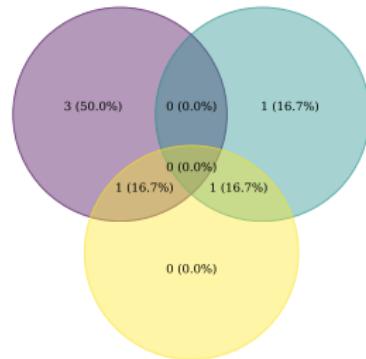
(d) Primary

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

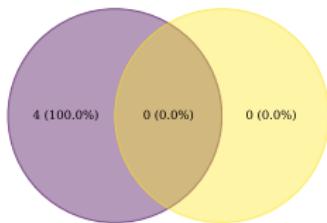
Peaks	AAH	AIS	Primary	CGC Genes
5p15.33			*	SDHA, TERT
7p11.1			*	
7p11.2	*			EGFR, ZNF479
11p11.11	*		*	
17p11.1	*			
18p11.1	*			
20p11.1	*			
20q13.33			*	PTK6, SS18L1

Peaks in LUAD III

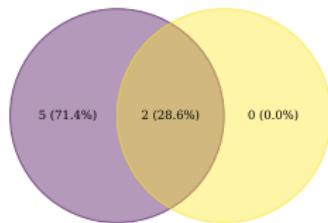
Table: Deletion Peaks in LUAD – stage

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

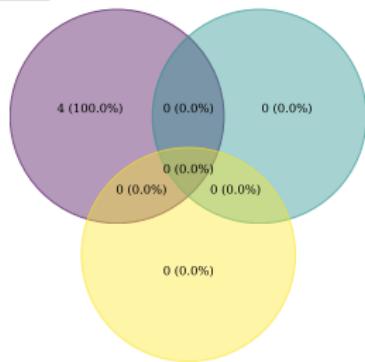
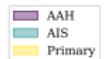
Precancer Peaks	Primary	CGC Genes
5p15.33	*	SDHA,TERT
7p11.1	*	
7p11.2	*	EGFR,ZNF479
11p11.11	*	*
18p11.1	*	
20p11.1	*	
20q13.33	*	PTK6,SS18L1

Peaks in LUAD VI

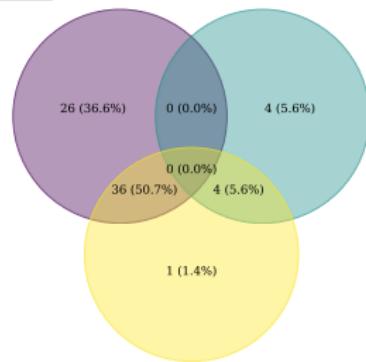
Table: Deletion Peaks in LUAD – PRE vs. PRI

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

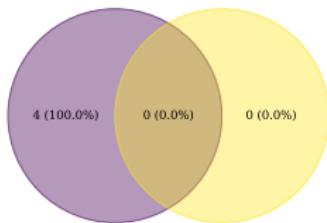
Genes	AAH	AIS	Primary
FRG1CP	*		
LOC644669	*		
LOC646813	*		
ZNF716	*		

Genes in LUAD III

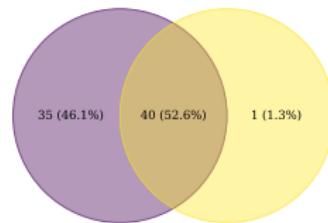
Table: Deletion Genes in LUAD – stage

Gene	AH	AG	Pivacy
ANKRD10A1	*	*	
ANKRD10B2	*		
ANKRD20A3	*	*	
ANKRD20A4		*	
AQP1P1	*		
AQP1P3		*	
ARHGAP3MP	*		
C5orf69			
CBWD6		*	
CFHD6	*		
CTNNAF1B			
COL11A1			
CTAG4A			
CTAG4B			
FAM20C			*
FAM20E2			
FAM44A1			*
FAM71A3			*
FAM74A4	*		
FAM84A2			
FAM98B1			*
FGF7P3			
FGF7P9			
FLJ10105			
FOXD4L4			*
FOXD4L4			*
FOXD4L4			*
FRG1HP			
GDI1P		*	
GLICR			
GYLYT1P1			
LCE3B			*
LCE3C			*
LCE3D			*
LCE3E			
LINC01189			*
LINC01140			*
LOC100930490			*
LOC100930327			
LOC100930195			*
LOC100930181			*
LOC100930683			
LOC100930683			*
LOC100930299			*
LOC100930298			*
LOC100930680			
LOC100930126			*
LOC100930686			
LOC100930684			
LOC39267			*
LOC44833			
LOC44896			
LOC50496			*
LOC50495			*
LOC738673			
MIR2169			
MIR3447A			*
OR2A1			
OR2A2P			
OR2A3P			
OR2A4P			*
OR4C11			
OR4C12			
OR4F1			
OR4F2			
OR4M2			
PTGERMP2	*		
PTGERMP2-CDKGAP3P2			
SPATA11A5			*
SPATA11A5			*
SPATA11A7			*
XLOC_207697	*		*
ZMPSTE			

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

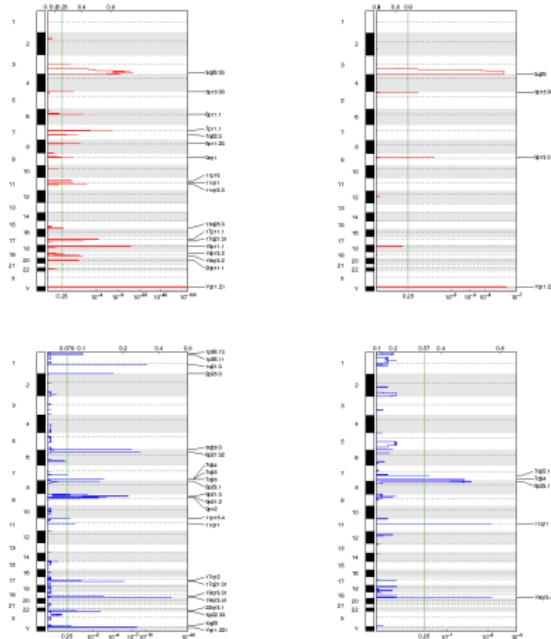
4.4.3. Gistic in Recurrence & LUSC

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	

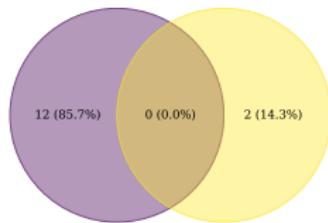
Gistic in Recurrence & LUSC – CIS



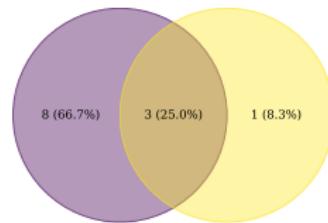
(a) Non-recurrence (b) Recurrence

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

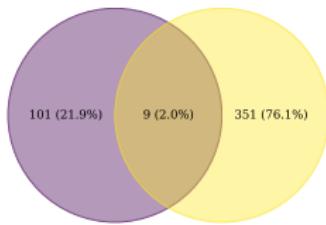
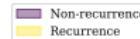
Peaks	Non-recurrence	Recurrence	CGC Genes
3q26.33	*		SOX2
3q29		*	MB21D2,MUC4,TFRC
5p15.33	*	*	SDHA,TERT
6p11.1	*		
7p11.1	*		
7q22.3	*		
8p11.23	*		FGFR1,NSD3
9p13.3		*	FANCG
9q11	*		
11p13	*		LMO2,WT1
11q11	*		
11q13.3	*		CCND1
15q25.3	*		NTRK3
17p11.1	*		
17q21.31	*		BRCA1,ETV4
18p11.1	*		
19p13.2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

Peaks in Recurrence & LUSC – CIS III

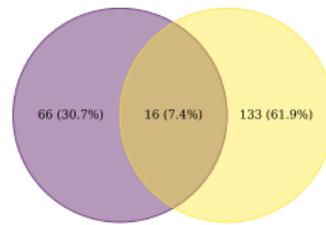
Table: Deletion Peaks in Recurrence & LUSC – CIS

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

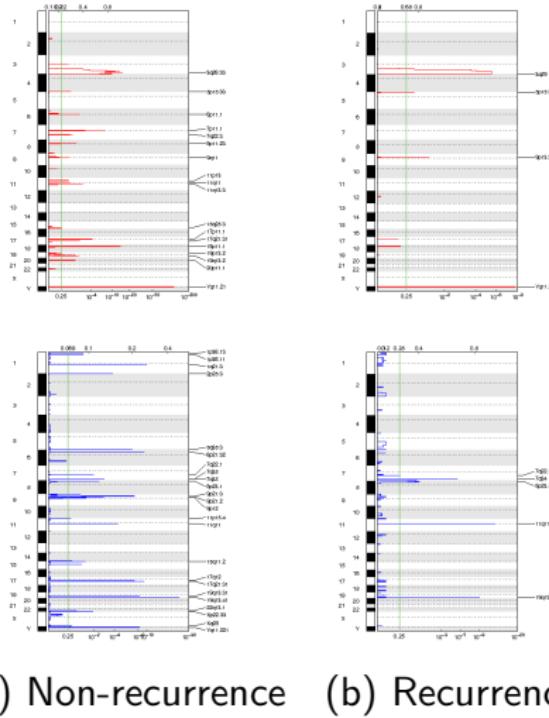
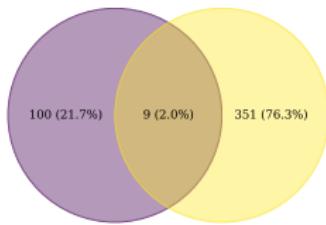
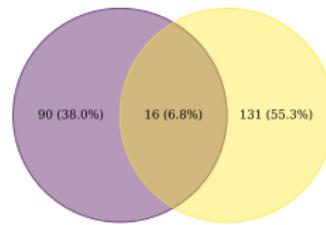


Figure: Gistic results in Recurrence & LUSC – Precancer

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

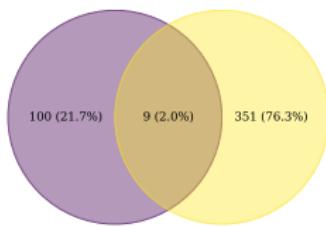
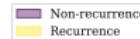
Peaks	Non-recurrence	Recurrence	CGC Genes
3q26.33	*		SOX2
3q29		*	MB21D2,MUC4,TFRC
5p15.33	*	*	SDHA,TERT
6p11.1	*		
7p11.1	*		
7q22.3	*		
8p11.23	*		FGFR1,NSD3
9p13.3		*	FANCG
9q11	*		
11p13	*		LMO2,WT1
11q11	*		
11q13.3	*		CCND1
15q25.3	*		NTRK3
17p11.1	*		
17q21.31	*		BRCA1,ETV4
18p11.1	*		
19p13.2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

Peaks in Recurrence & LUSC – Precancer III

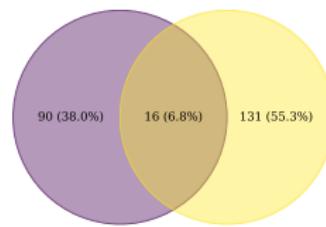
Table: Deletion Peaks in Recurrence & LUSC – Precancer

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

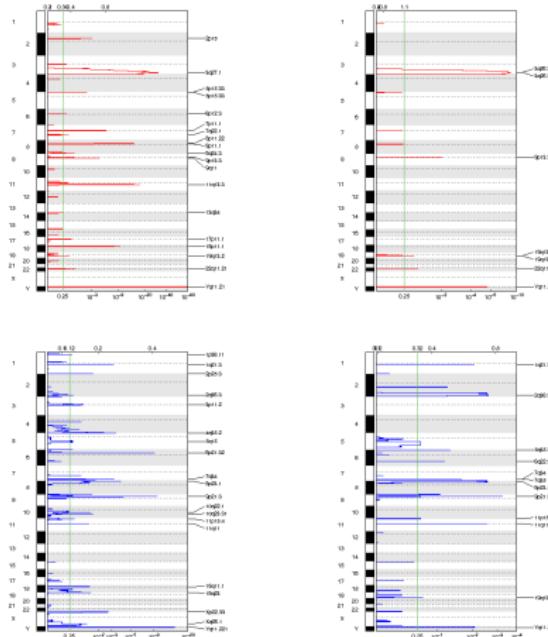
CGC Genes	Non-recurrence	Recurrence
BCL6		*
CCND1	*	
EIF4A2		*
ETV5		*
FANCG		*
FGFR1	*	
IGF2BP2		*
LPP		*
MAP3K13		*
MB21D2		*
MECOM		*
MUC4		*
PIK3CA		*
SOX2	*	*
TBL1XR1		*
TFRC		*
TP63		*

Genes in Recurrence & LUSC – Precancer III

Table: Deletion Genes in Recurrence & LUSC – Precancer

CGC Genes	Non-recurrence	Recurrence
ARHGEF10		*
CDKN2A	*	

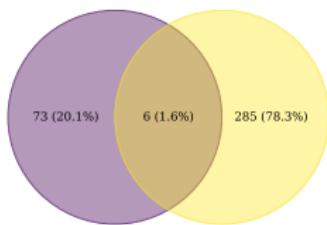
Gistic in Recurrence & LUSC – Primary



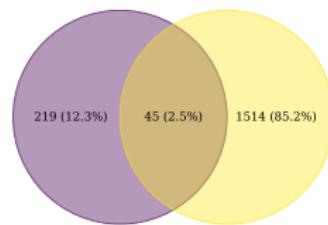
(a) Non-recurrence (b) Recurrence

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

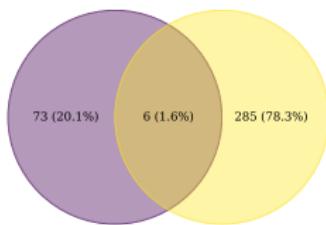
Peaks	Non-recurrence	Recurrence	CGC Genes
2p15	*		XPO1
3q26.33		*	SOX2
3q27.1	*		
5p15.33	*		SDHA,TERT
6p12.3	*		
7p11.1	*		
7q22.1	*		CUX1,TRRAP
8p11.1	*		
8p11.22	*		
8q24.3	*		RECQL4
9p13.3	*	*	FANCG
9q11	*		
11q13.3	*		CCND1
13q34	*		
17p11.1	*		
18p11.1	*		
19q13.2	*	*	AKT2,CD79A,CIC
22q11.21	*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

Peaks in Recurrence & LUSC – Primary III

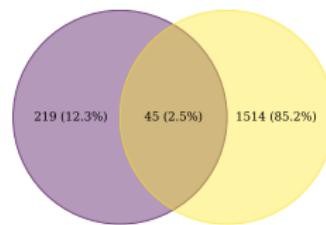
Table: Deletion Peaks in Recurrence & LUSC – Primary

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	*	
ITGA4	*	
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

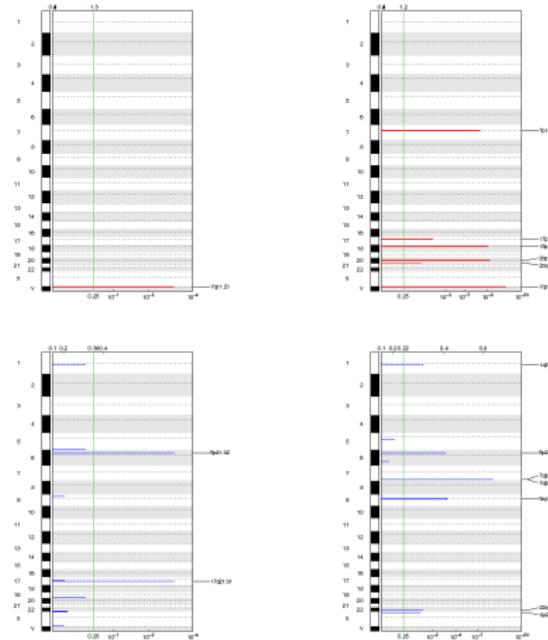
4.4.4. Gistic in Recurrence & LUAD

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

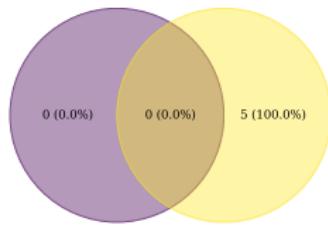
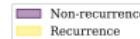
Gistic in Recurrence & LUAD – AAH



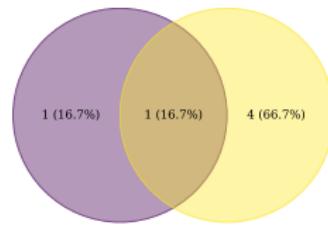
(a) Non-recurrence (b) Recurrence

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

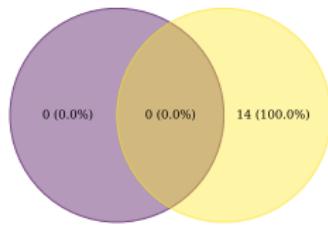
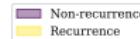
Peaks	Non-recurrence	Recurrence	CGC Genes
7p11.1		*	
17p11.1		*	
18p11.1		*	
20p11.1		*	
20q13.33		*	PTK6,SS18L1

Peaks in Recurrence & LUAD – AAH III

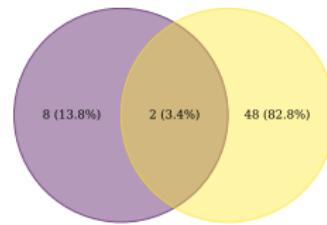
Table: Deletion Peaks in Recurrence & LUAD – AAH

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

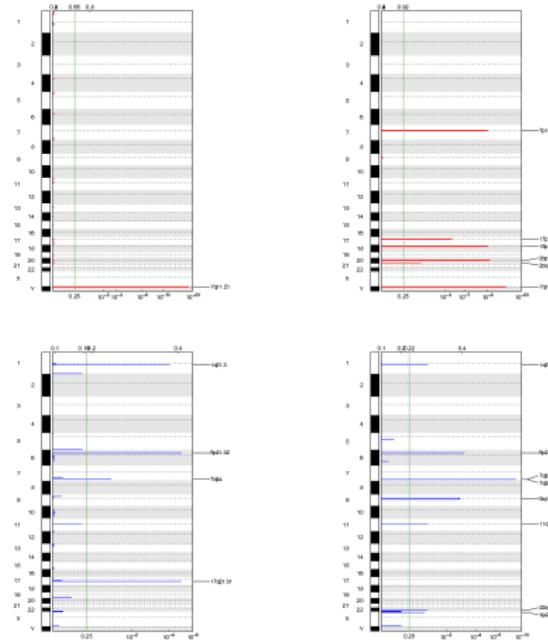
Genes	Non-recurrence	Recurrence
C20orf197		*
CDH26		*
CDH4		*
FRG1CP		*
LOC100506470		*
LOC101928048		*
LOC644669		*
LOC729296		*
MIR4533		*
MIR548AG2		*
MIR646		*
MIR646HG		*
MTRNR2L1		*
ZNF716		*

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	*	
Cblf10		*
CBWD5		*
CTAGE4		*
CTAGE8		*
FAM27C		*
FAM74A1		*
FAM74A4		*
FAM98B1		*
FGF19P6		*
FLJ4315		*
FOOD44		*
FOOD45		*
FRG1LP		*
GXYLT1P3		*
HCG23		*
LCE3A		*
LCE3B		*
LINC01180		*
LINC01410		*
LOC100132249		*
LOC1019203195		*
LOC1019203831		*
LOC1019203983		*
LOC1019209983		*
LOC102723709		*
LOC102724238		*
LOC1027246905		*
LOC38097		*
LOC403323		*
LOC554249		*
LOC554269		*
LOC5542873		*
LRRK3TA		*
LRRK3TA2		*
MIR4427A		*
NSF	*	
NSF1	*	
OR2A1	*	
OR2A2P		*
OR2A7		*
OR2A9P		*
PTENAMP2-CDK2AP2P2		*
SPATA11A5		*
SPATA11A7		*
XLOC_007897		*
ZNF658		*

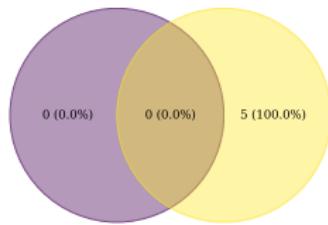
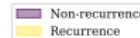
Gistic in Recurrence & LUAD – Precancer



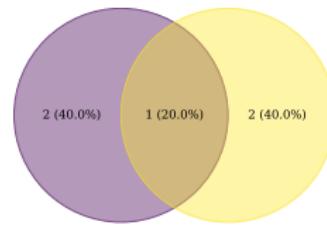
(a) Non-recurrence (b) Recurrence

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

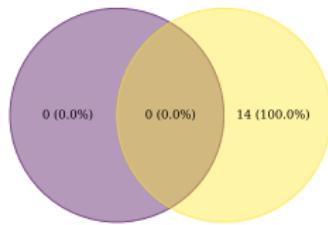
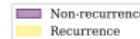
Peaks	Non-recurrence	Recurrence	CGC Genes
7p11.1		*	
17p11.1		*	
18p11.1		*	
20p11.1		*	
20q13.33		*	PTK6,SS18L1

Peaks in Recurrence & LUAD – Precancer III

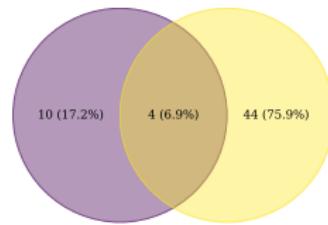
Table: Deletion Peaks in Recurrence & LUAD – Precancer

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

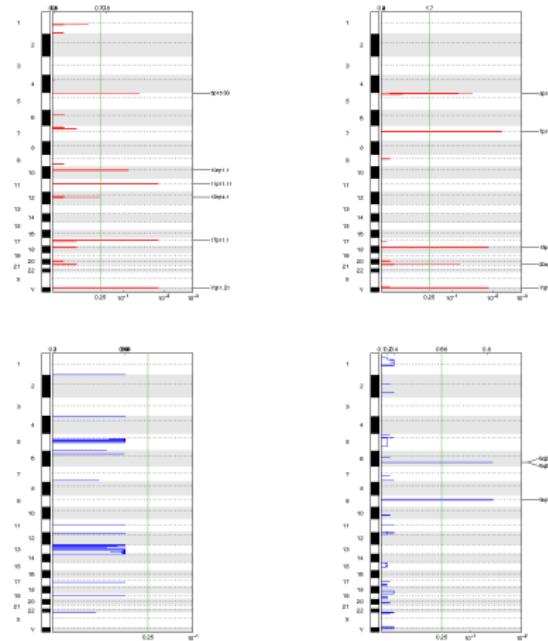
Genes	Non-recurrence	Recurrence
C20orf197		*
CDH26		*
CDH4		*
FRG1CP		*
LOC100506470		*
LOC101928048		*
LOC644669		*
LOC729296		*
MIR4533		*
MIR548AG2		*
MIR646		*
MIR646HG		*
MTRNR2L1		*
ZNF716		*

Genes in Recurrence & LUAD – Precancer III

Table: Deletion Genes in Recurrence & LUAD – Precancer

Genes	Non-recurrence	Recurrence
ANKRD20A1	*	
ANKRD20A3	*	
ANKRD20A4	*	
AQP1P1	*	
AQP1P3	*	
ARHGEF34P	*	
ARL17A	*	
ARL17B	*	
BTNL2	*	*
C6orf10		
CBWD5		
CTAGE4		
CTSL2B		
FAM27C		
FAM74A1		
FAM74A3		
FAM74A4		
FAM74B1		
FGF19		
FLJ4315		
FOXDML4		
FOXDML5		
FRLG1B	*	
GNA1TP3		
HCG23		*
LCE1A	*	
LCE1B	*	
LCE1C	*	
LCE1D	*	
LCE1E	*	
LINC01189		
LINC01410		
LOC10013249		
LOC100132935		
LOC101920381		
LOC101920913	*	
LOC101920983		
LOC101923709		
LOC101924238		
LOC101924695		
LOC286297		
LOC403323		
LOC554249		
LOC642269		
LOC722873		
LRRK3TA		
LRRK3TA2		
MIR4477A		
NSP1P1		
OR2A24		
OR2A26P		
OR2A27		
OR2A28P		
PTENAMP2-CDK2AP2P2		
SPTA11A5		
SPTA11A7		
XLOC_007897		
ZNF658	*	

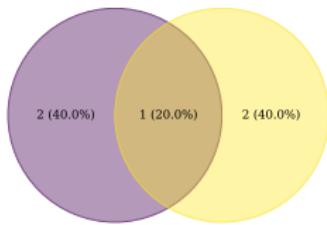
Gistic in Recurrence & LUAD – Primary



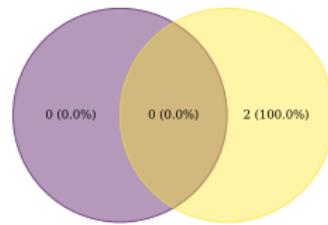
(a) Non-recurrence (b) Recurrence

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

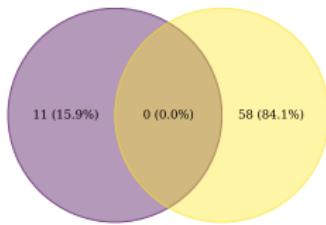
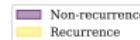
Peaks	Non-recurrence	Recurrence	CGC Genes
5p15.33	*	*	SDHA, TERT
7p11.1		*	
10q11.1	*		
11p11.11	*		
12q14.1	*		CDK4, LRIG3
17p11.1	*		
18p11.1		*	
20q13.33		*	PTK6, SS18L1

Peaks in Recurrence & LUAD – Primary III

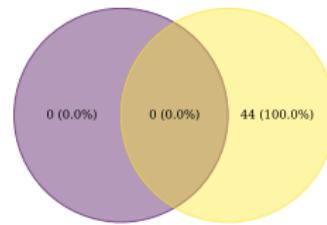
Table: Deletion Peaks in Recurrence & LUAD – Primary

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

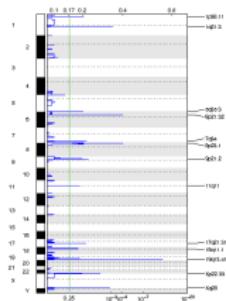
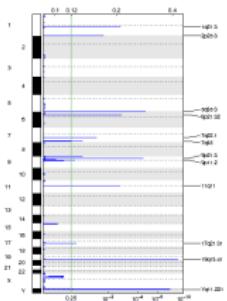
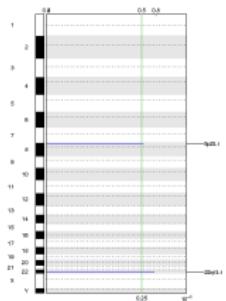
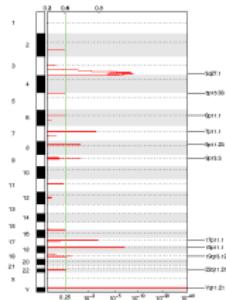
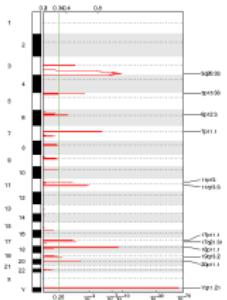
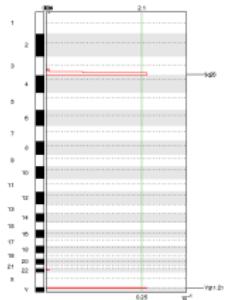
4.4.5. Gistic in Smoking & LUSC

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	

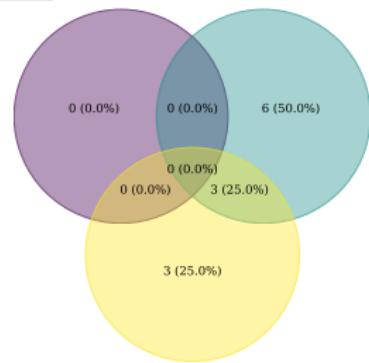
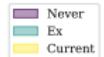
Gistic in Smoking & LUSC – CIS



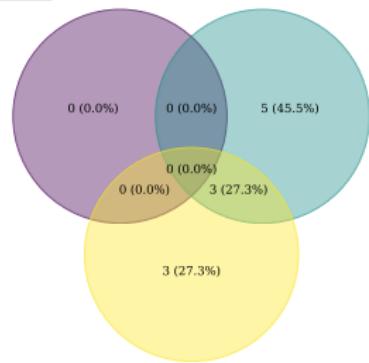
(a) Never Smoker (b) Ex-Smoker (c) Current Smoker

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

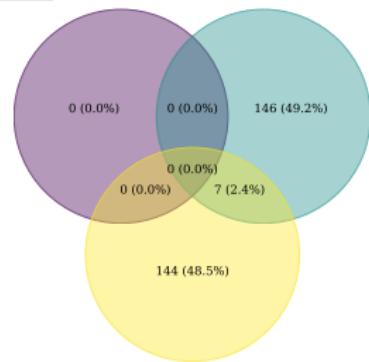
Peaks	Never	Ex	Current	CGC Genes
3q26.33		*		SOX2
3q27.1			*	
3q28	*			LPP,TP63
5p15.33		*	*	SDHA,TERT
6p11.1			*	
6p12.3		*		
7p11.1		*	*	
8p11.23			*	FGFR1,NSD3
9p13.3			*	FANCG
11p13		*		LMO2,WT1
11q13.3		*		CCND1
17p11.1		*	*	
17q21.31		*		BRCA1,ETV4
18p11.1		*	*	
19q13.2		*		AKT2,CD79A,CIC
19q13.12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

Peaks in Smoking & LUSC – CIS III

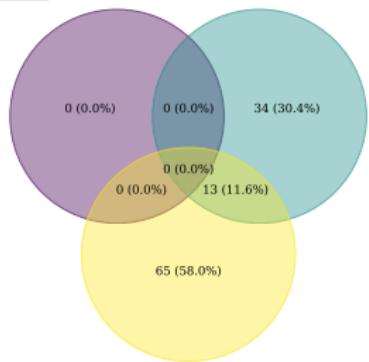
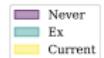
Table: Deletion Peaks in Smoking & LUSC – CIS

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

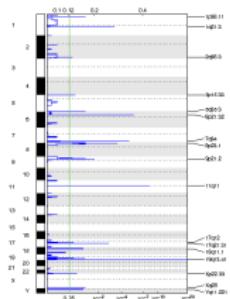
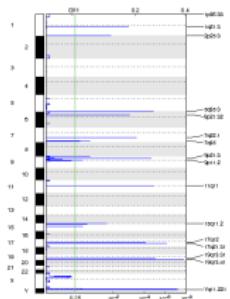
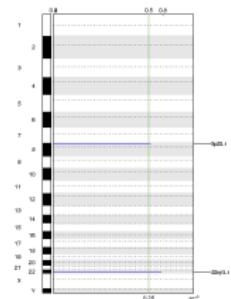
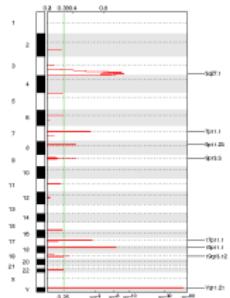
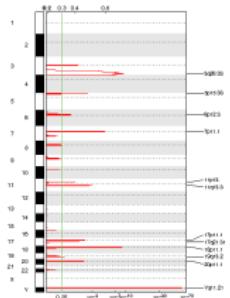
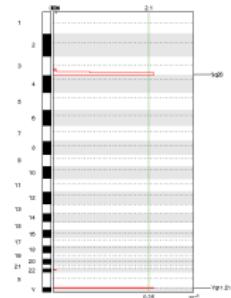
CGC Genes	Never	Ex	Current
ANK1			*
CCND1		*	
EXT2		*	
FANCG			*
FGFR1			*
HOOK3			*
IKBKB			*
KAT6A			*
LMO2		*	
PAX5			*
SOX2		*	
WT1		*	

Genes in Smoking & LUSC – CIS III

Table: Deletion Genes in Smoking & LUSC – CIS

CGC Genes	Never	Ex	Current
CDKN2A		*	*
MLLT3			*

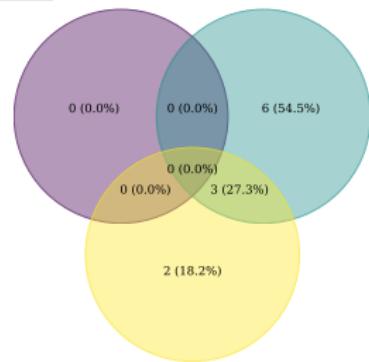
Gistic in Smoking & LUSC – Precancer



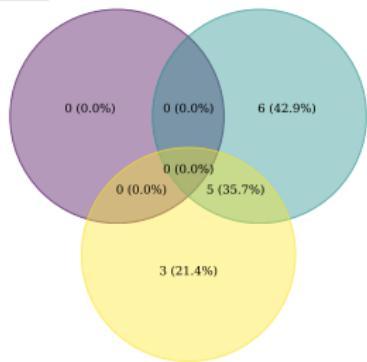
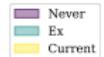
(a) Never Smoker (b) Ex-Smoker (c) Current Smoker

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

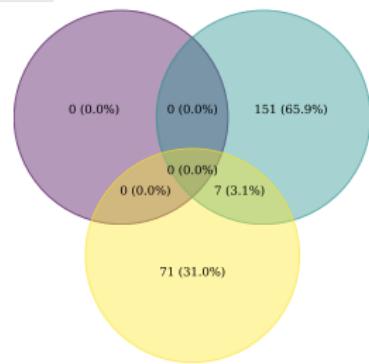
Peaks	Never	Ex	Current	CGC Genes
3q26.33		*		SOX2
3q27.1			*	
3q28	*			LPP,TP63
5p15.33		*		SDHA,TERT
6p12.3		*		
7p11.1	*		*	
8p11.23			*	FGFR1,NSD3
9p13.3			*	FANCG
11p13		*		LMO2,WT1
11q13.3		*		CCND1
17p11.1	*		*	
17q21.31		*		BRCA1,ETV4
18p11.1	*		*	
19q13.2	*			AKT2,CD79A,CIC
19q13.12			*	
20p11.1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

Peaks in Smoking & LUSC – Precancer III

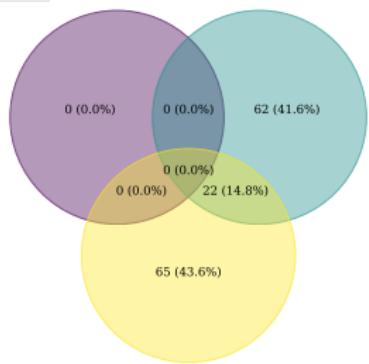
Table: Deletion Peaks in Smoking & LUSC – Precancer

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

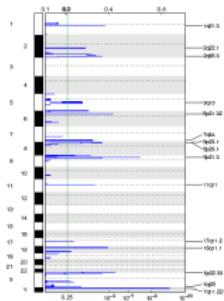
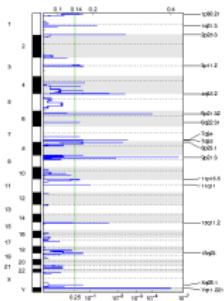
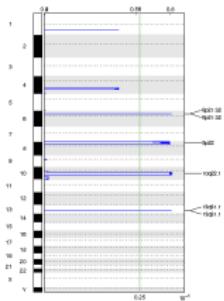
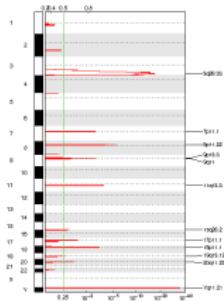
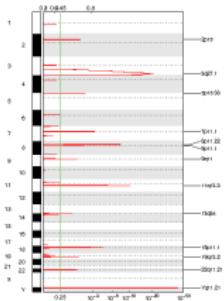
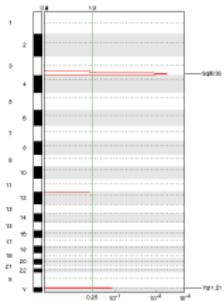
CGC Genes	Never	Ex	Current
CCND1		*	
EXT2		*	
FANCG			*
LMO2		*	
PAX5			*
SOX2		*	
WT1		*	

Genes in Smoking & LUSC – Precancer III

Table: Deletion Genes in Smoking & LUSC – Precancer

CGC Genes	Never	Ex	Current
CDKN2A	*	*	
MLLT3		*	

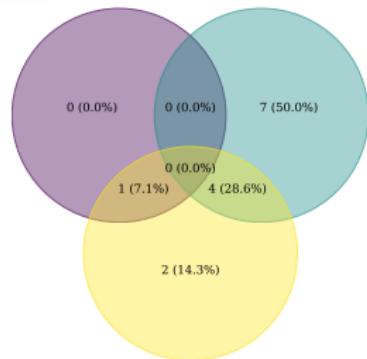
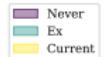
Gistic in Smoking & LUSC – Primary



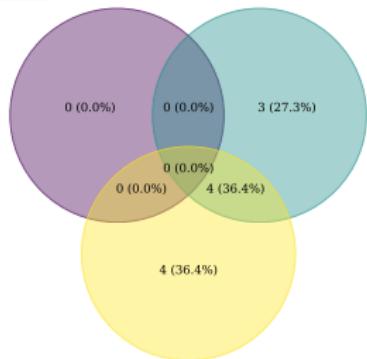
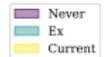
(a) Never Smoker (b) Ex-Smoker (c) Current Smoker

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

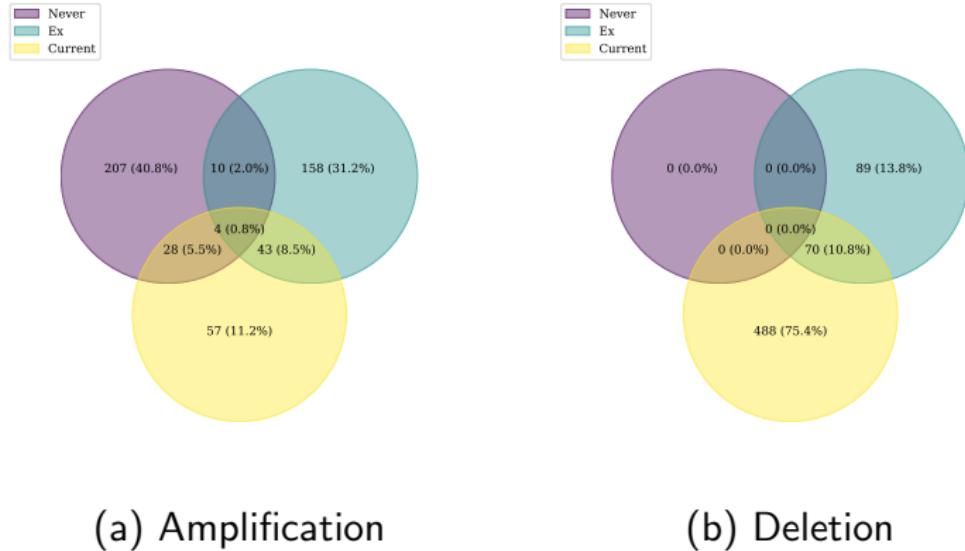


Figure: Venn Diagram among Genes in Smoking & LUSC – Primary

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

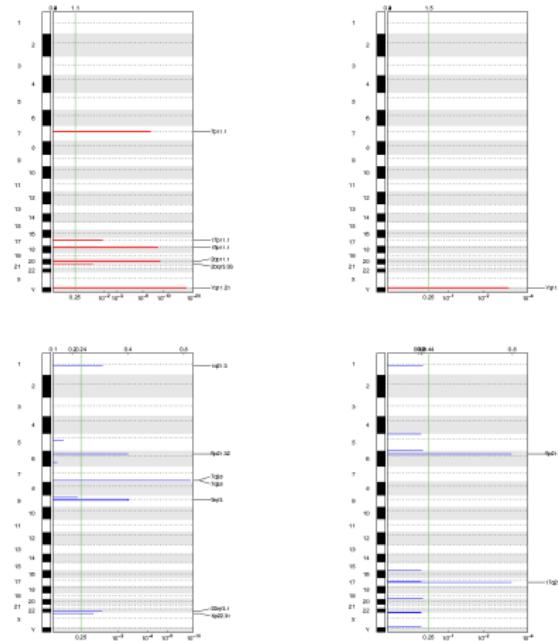
4.4.6. Gistic in Smoking & LUAD

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	

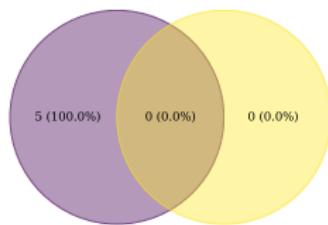
Gistic in Smoking & LUAD – AAH



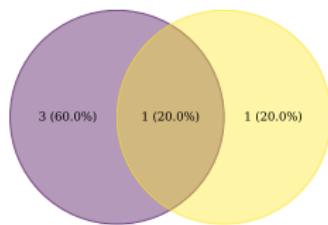
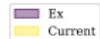
(a) Ex-Smoker (b) Current Smoker

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

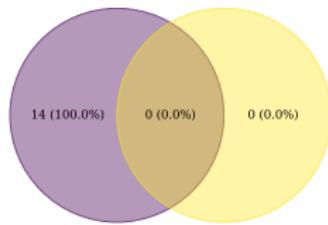
Peaks	Ex	Current	CGC Genes
7p11.1	*		
17p11.1	*		
18p11.1	*		
20p11.1	*		
20q13.33	*		PTK6,SS18L1

Peaks in Smoking & LUAD – AAH III

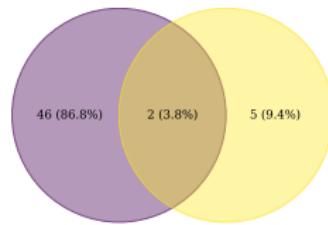
Table: Deletion Peaks in Smoking & LUAD – AAH

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

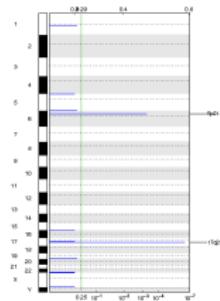
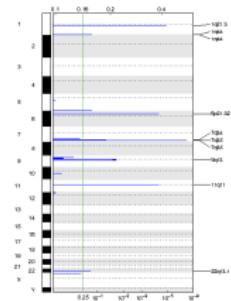
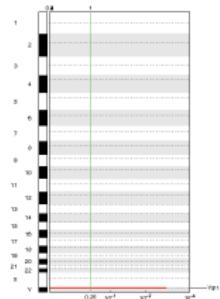
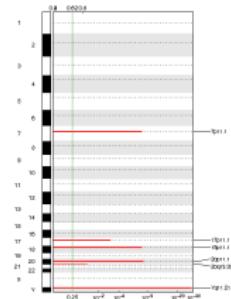
Genes	Ex	Current
C20orf197	*	
CDH26	*	
CDH4	*	
FRG1CP	*	
LOC100506470	*	
LOC101928048	*	
LOC644669	*	
LOC729296	*	
MIR4533	*	
MIR548AG2	*	
MIR646	*	
MIR646HG	*	
MTRNR2L1	*	
ZNF716	*	

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

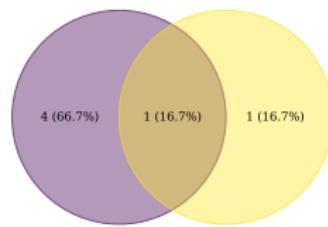
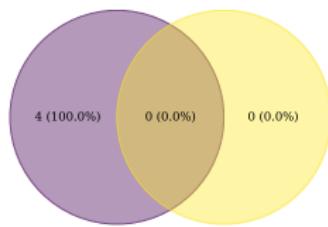


(a) Ex-Smoker

(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

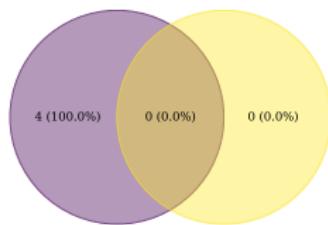
Peaks	Ex	Current	CGC Genes
7p11.1	*		
17p11.1	*		
18p11.1	*		
20p11.1	*		
20q13.33	*		PTK6,SS18L1

Peaks in Smoking & LUAD – Precancer III

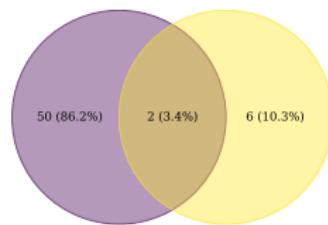
Table: Deletion Peaks in Smoking & LUAD – Precancer

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

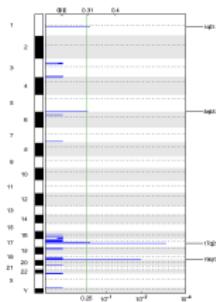
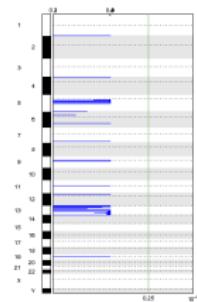
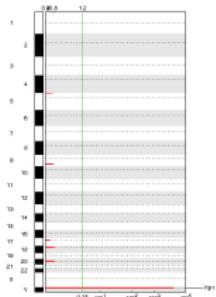
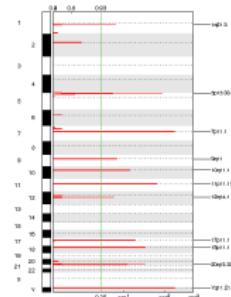
Genes	Ex	Current
FRG1CP	*	
LOC644669	*	
MTRNR2L1	*	
ZNF716	*	

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		
lnc01189		
lnc01410		
LOC10013249		
LOC101981995		
LOC101982001		
LOC101983183		
LOC101985833		
LOC102723709		
LOC102742420		
LOC103980005		
LOC26027		
LOC403323		
LOC594249		
LOC642029		
LOC729973		
LRRK3TA		
LRRK3TA2		
MIR4477A		
NSFP1		
O84241		
O84246P		
O84247		
O84248P		
O84C11		
O84E5		
O84H4		
O84S2		
PTGER4P2-CDK2AP2P2	*	
SPATA31A5		
SPATA31A7		
XLOC_097647		
ZNF658	*	

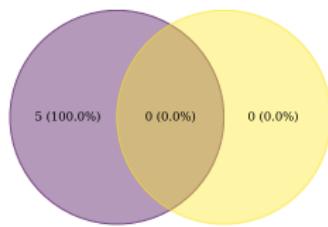
Gistic in Smoking & LUAD – Primary



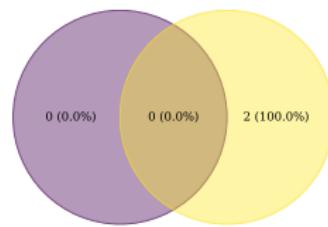
(a) Ex-Smoker (b) Current Smoker

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

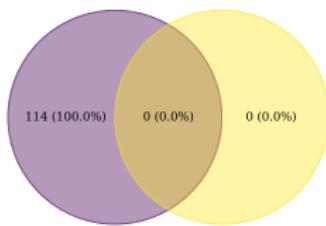
Peaks	Ex	Current	CGC Genes
1q21.3	*		ARNT,MLLT11,S100A7,SETDB1,TPM3
5p15.33	*		SDHA,TERT
7p11.1	*		
9q11	*		
10q11.1	*		
11p11.11	*		
12q14.1	*		CDK4,LRIG3
17p11.1	*		
18p11.1	*		
20q13.33	*		PTK6,SS18L1

Peaks in Smoking & LUAD – Primary III

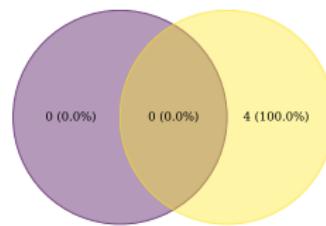
Table: Deletion Peaks in Smoking & LUAD – Primary

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?

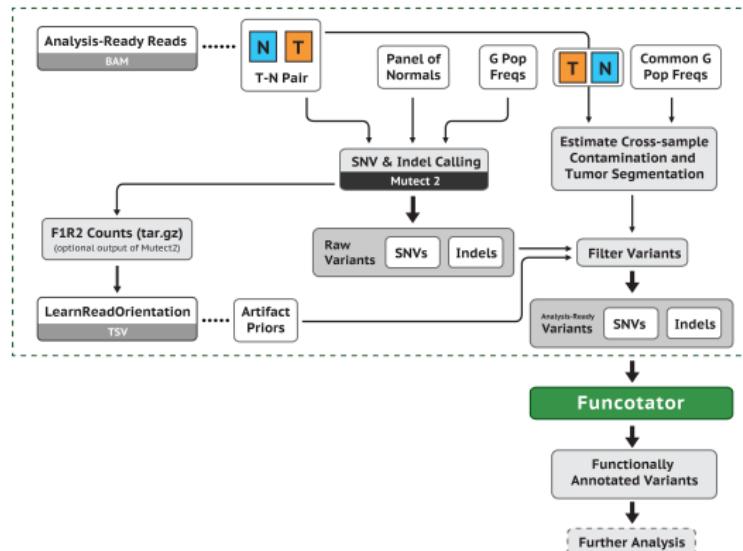
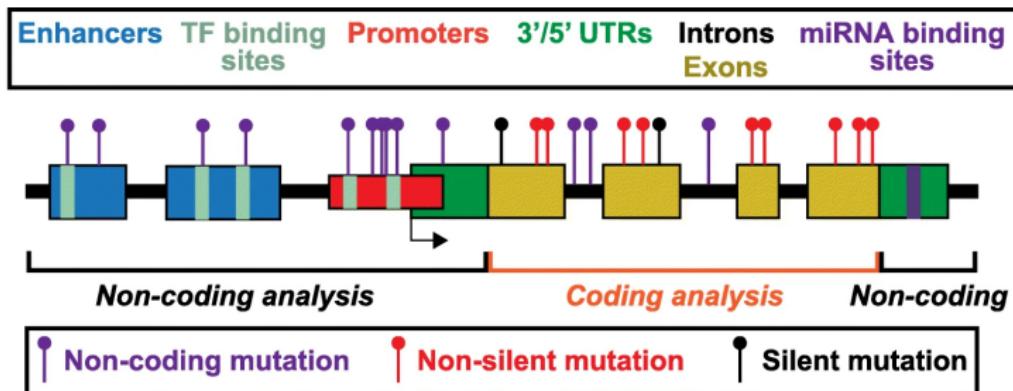


Figure: Somatic short variant discovery workflow (Van der Auwera et al., 2013; DePristo et al., 2011)

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?

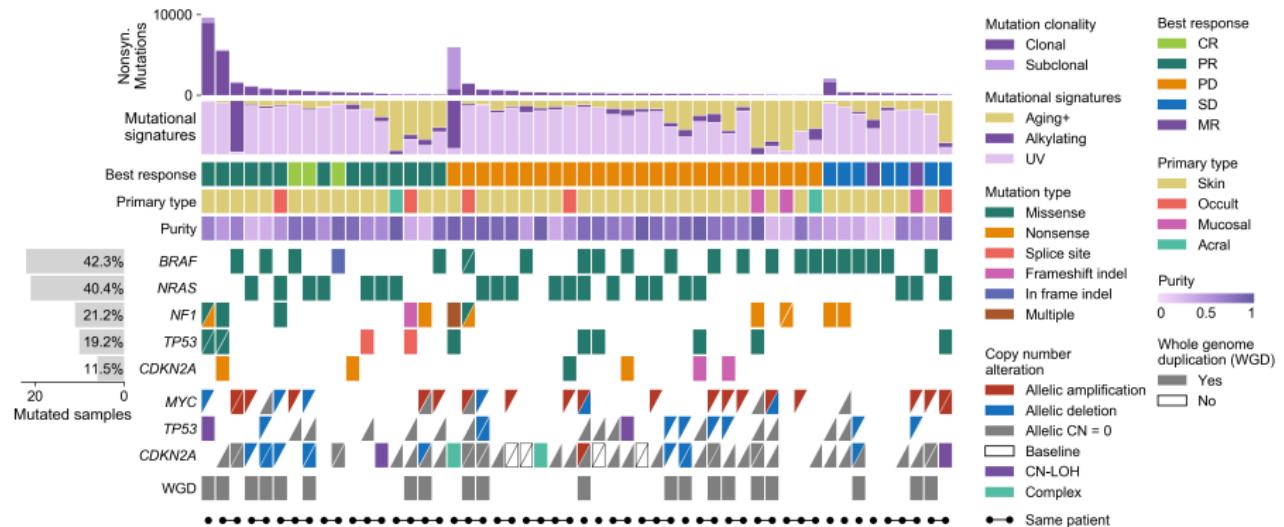


Figure: A comutation plot generated with CoMut (Crowdis et al., 2020)

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

4. Results

4.5. Single Nucleotide Variations Analysis

4.5.1. Somatic Variant

Somatic Variant in LUSC

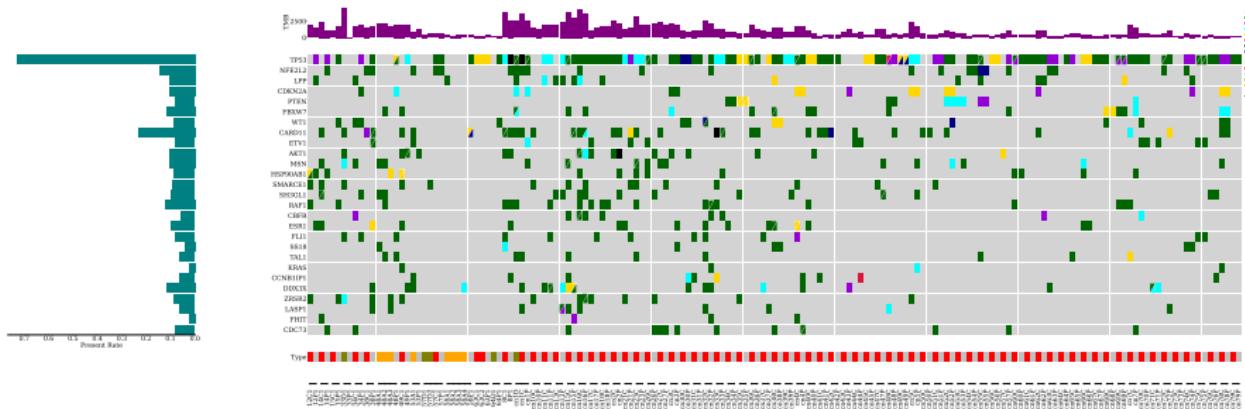


Figure: CoMut Plot with LUSC Patients

Somatic Variant in LUAD

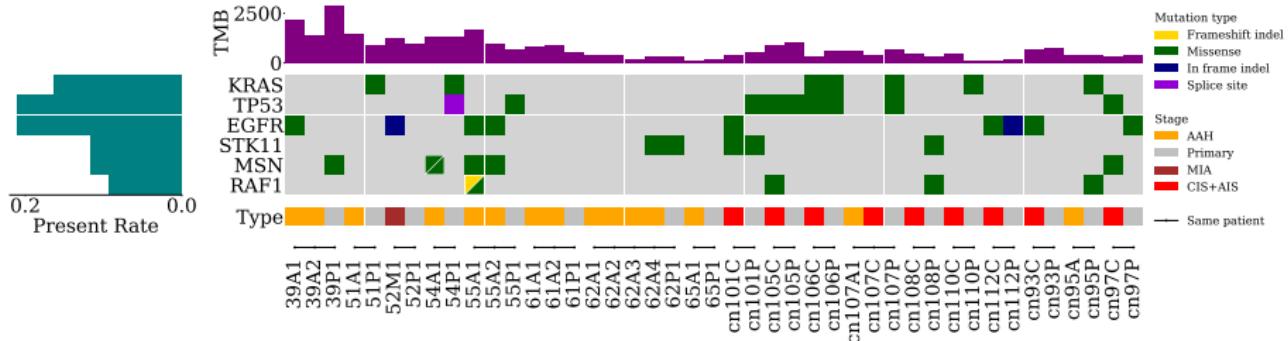


Figure: CoMut Plot with LUAD Patients

4. Results

4.5. Single Nucleotide Variations Analysis

4.5.2. Somatic Variant with Recurrence

Somatic Variant in LUSC with Recurrence

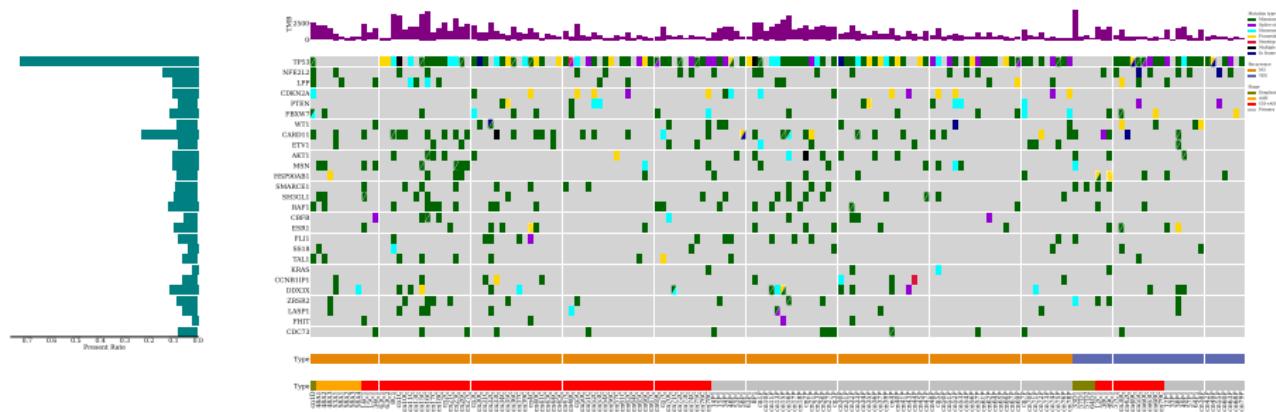


Figure: CoMut Plot in LUSC Patients with Recurrence

Somatic Variant in LUAD with Recurrence

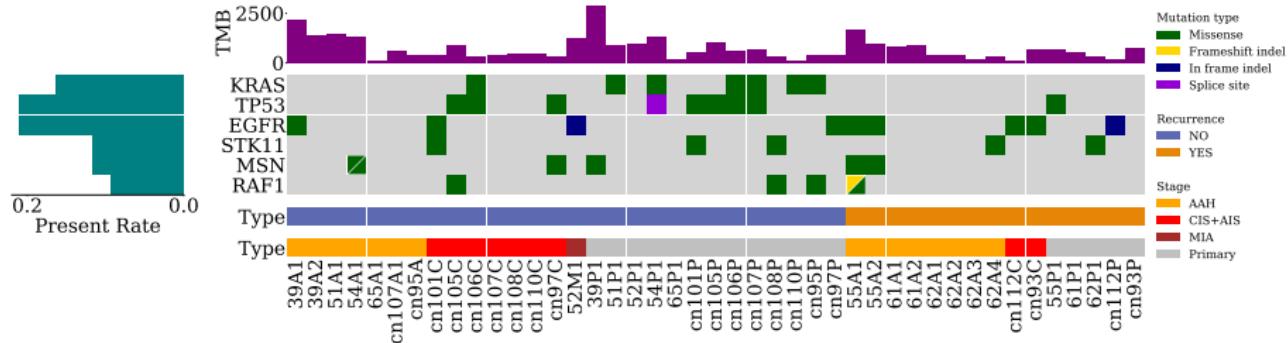


Figure: CoMut Plot in LUAD Patients with Recurrence

4. Results

4.5. Single Nucleotide Variations Analysis

4.5.3. Somatic Variant with Smoking

Somatic Variant in LUSC with Smoking

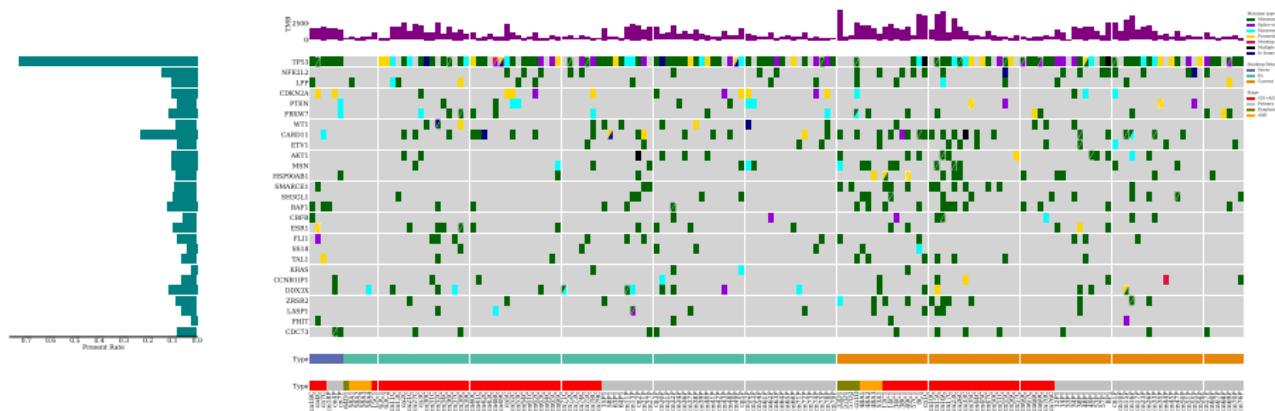


Figure: CoMut Plot in LUSC Patients with Smoking

Somatic Variant in LUAD with Smoking

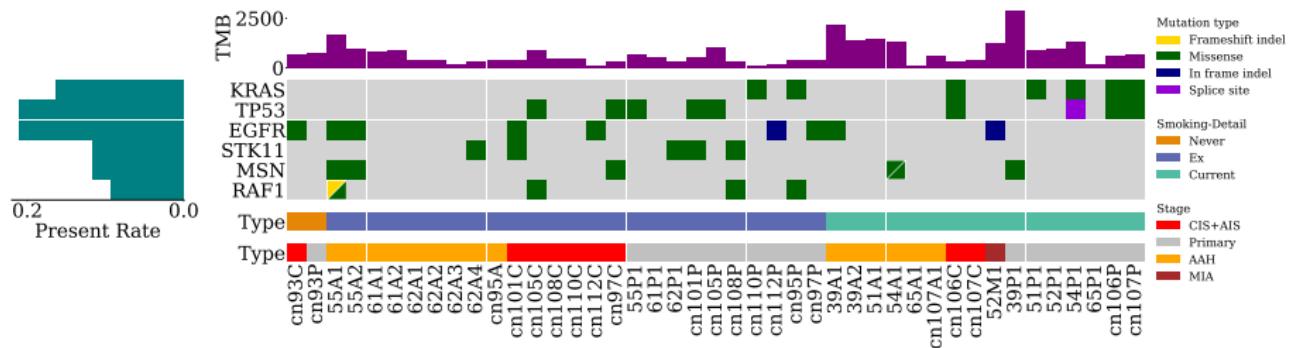


Figure: CoMut Plot in LUAD Patients with Smoking

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

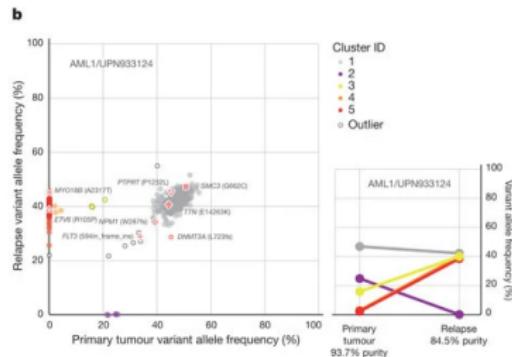
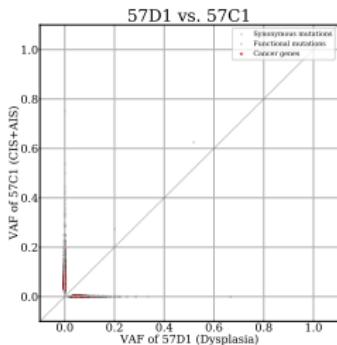
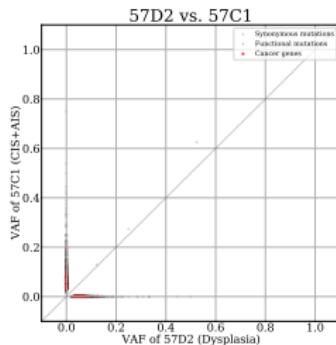


Figure: VAF distribution of validated mutations (L. Ding et al., 2012)

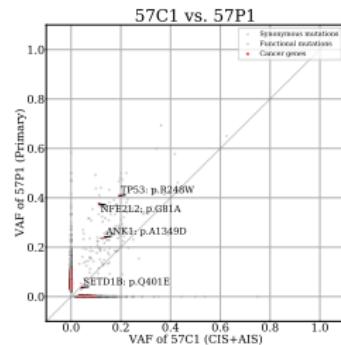
VAF Plots



(a) Dysplasia + CIS



(b) Dysplasia + CIS



(c) CIS + Primary

Figure: VAF plots in patient #57

PyClone?

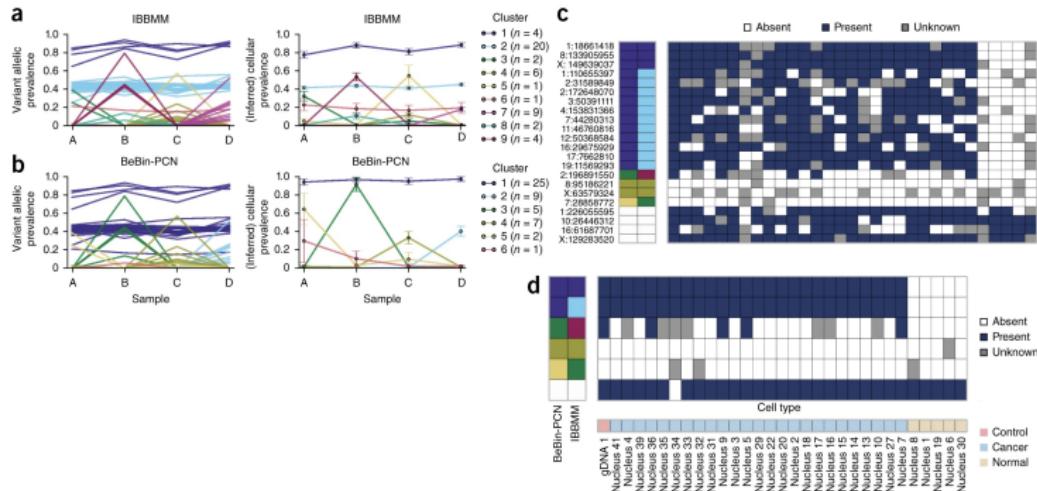
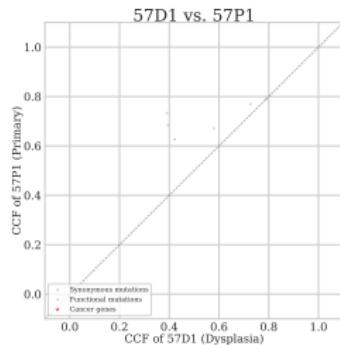
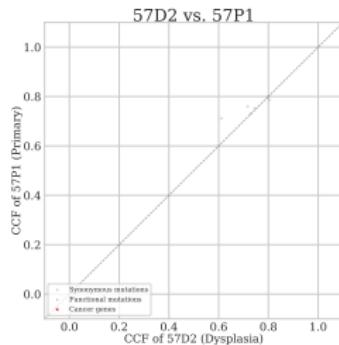


Figure: Analysis of multiple samples by PyClone (Roth et al., 2014)

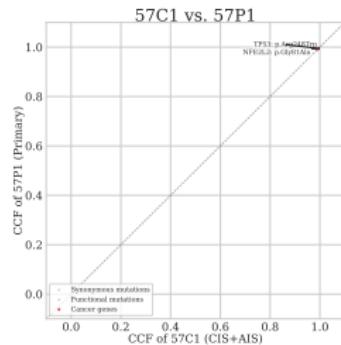
PyClone Plots I



(a) 57D1



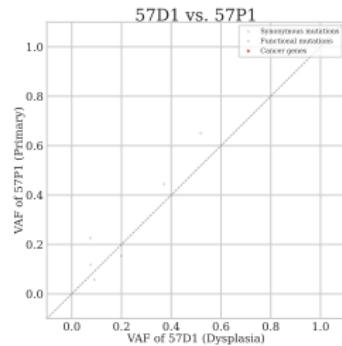
(b) 57D2



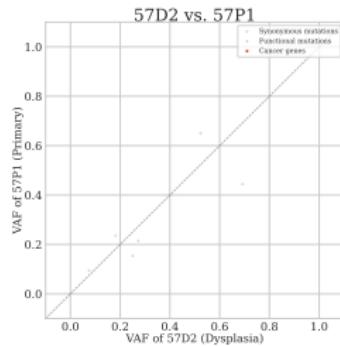
(c) 57C1

Figure: CCF plot in patient #57

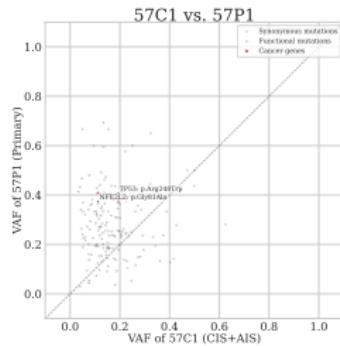
PyClone Plots II



(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?

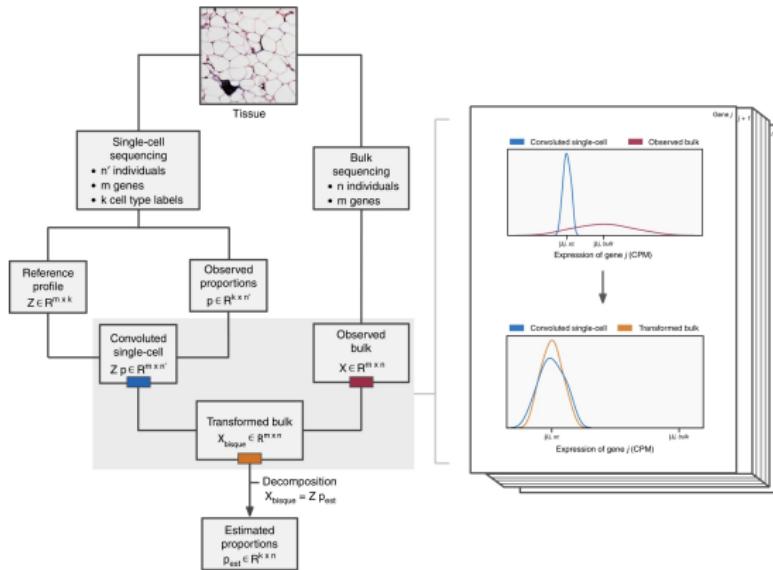


Figure: Workflow for BisqueRNA (Jew et al., 2020)

4. Results

4.8. Bulk Cell Deconvolution

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

Reference Single-cell Data

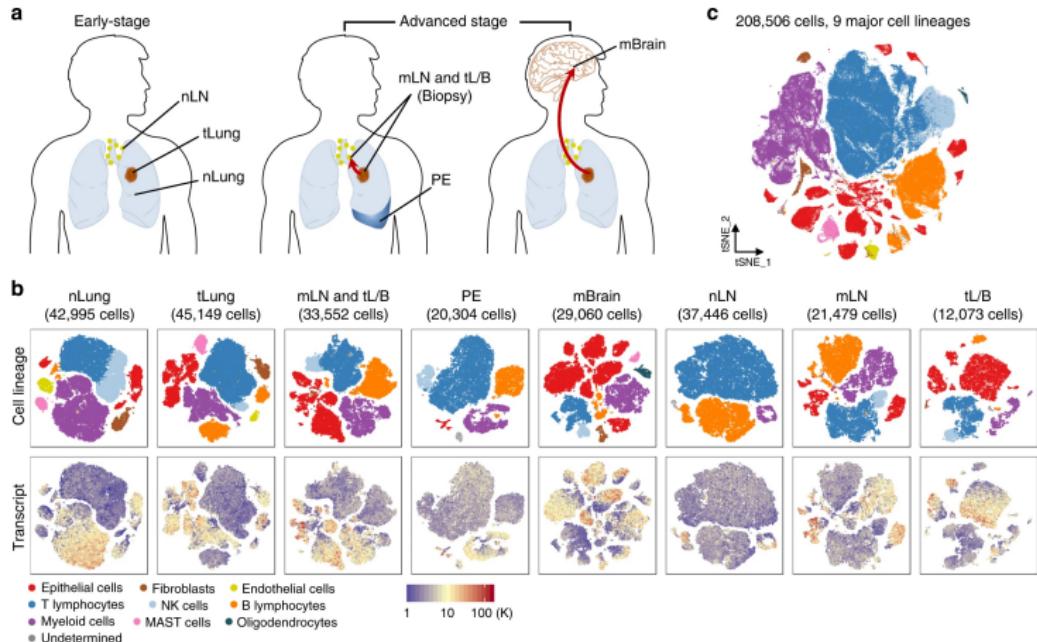


Figure: Comprehensive dissection and clustering of 208,506 single cells from LUAD patients (N. Kim et al., 2020)

Cluster Plot in LUSC

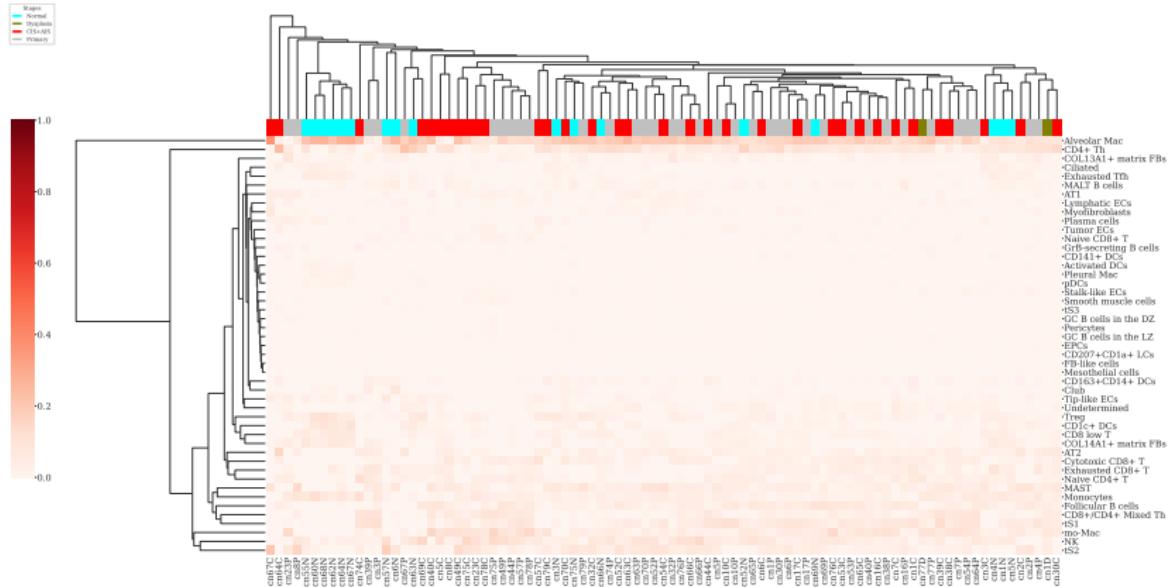
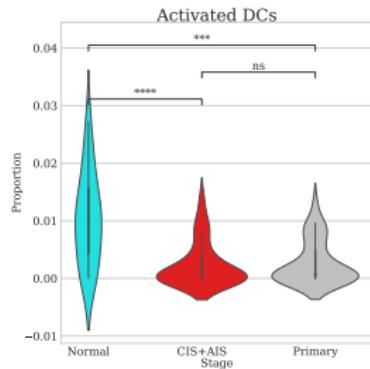
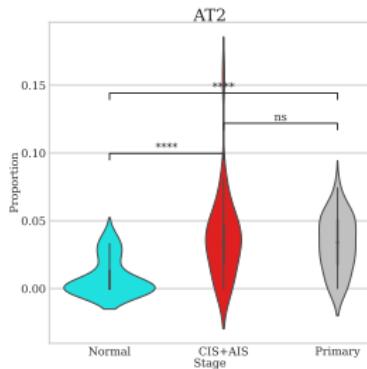


Figure: Cluster Plot in LUSC

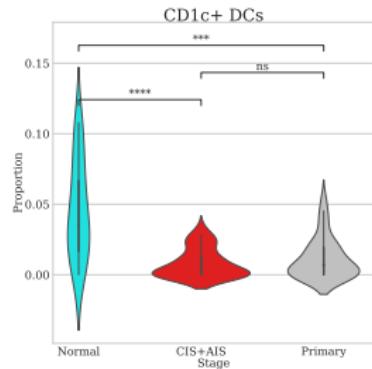
Violin Plots in LUSC I



(a) Activated DCs



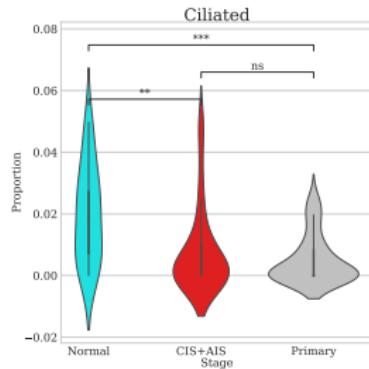
(b) Alveolar type II



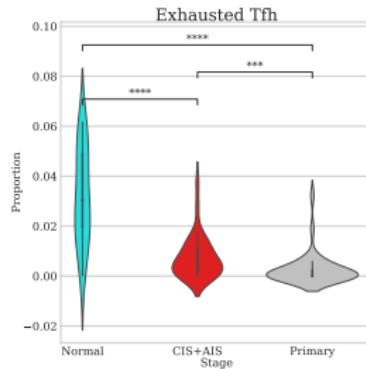
(c) Langerhans cells

Figure: Violin Plots in LUSC

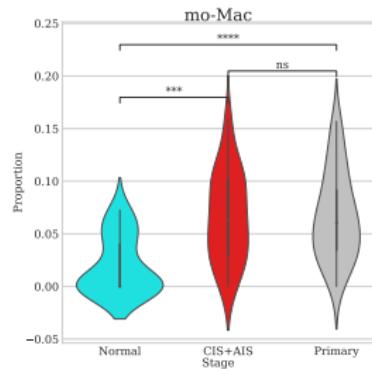
Violin Plots in LUSC II



(d) Ciliated cells



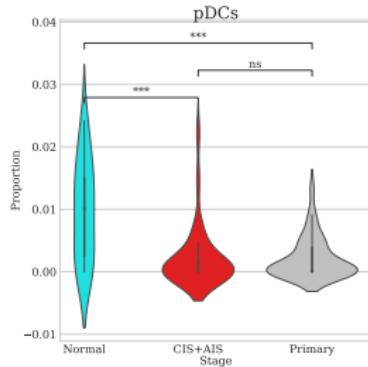
(e) Exhausted T follicular helper



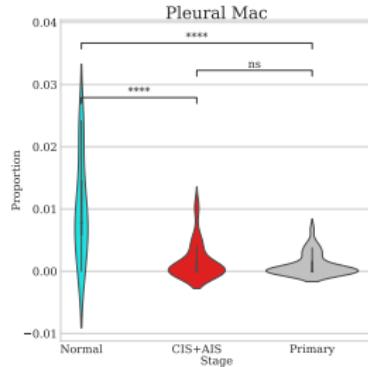
(f) Mo & Mac

Figure: Violin Plots in LUSC

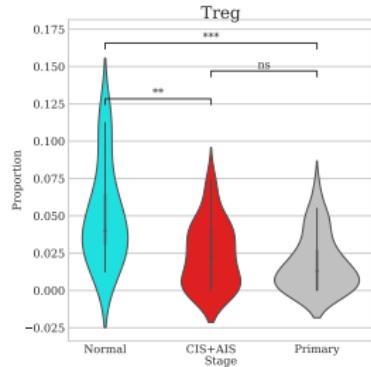
Violin Plots in LUSC III



(g) Plasmacytoid DCs



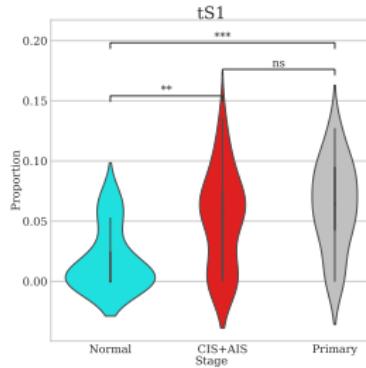
(h) Pleural Mac



(i) Regulatory T cells

Figure: Violin Plots in LUSC

Violin Plots in LUSC IV



(j) Transcriptional states 1

Figure: Violin Plots in LUSC

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

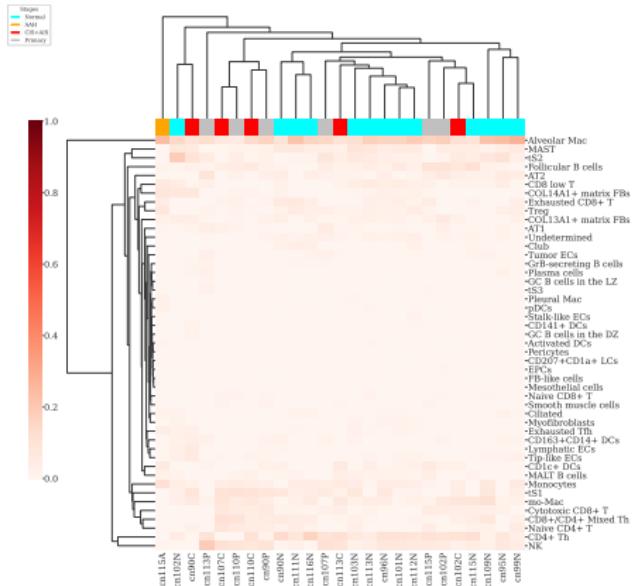
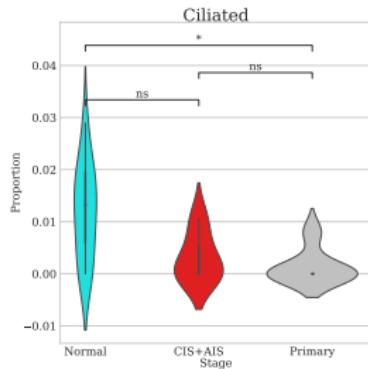
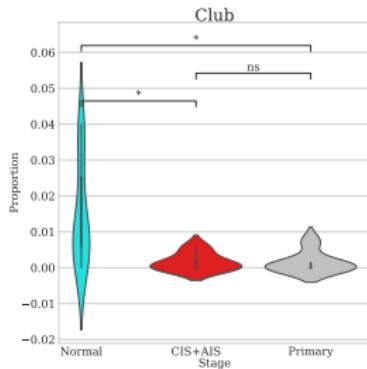


Figure: Cluster Plot in LUAD

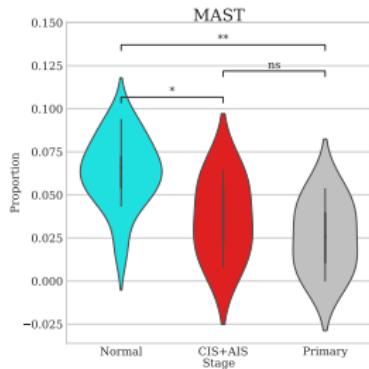
Violin Plots in LUAD I



(a) Ciliated cells



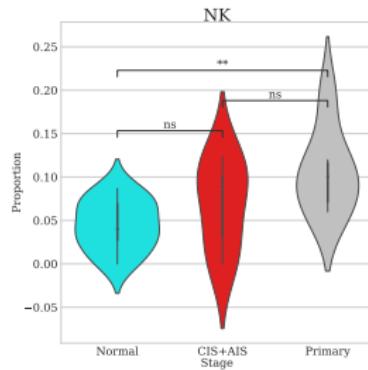
(b) Club Cell



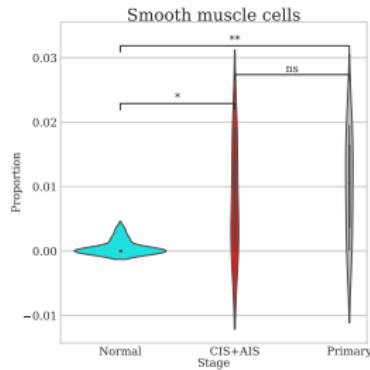
(c) Mast cell

Figure: Violin Plots in LUAD

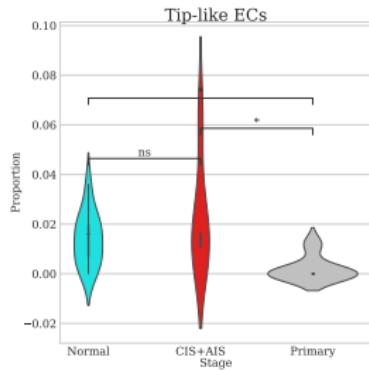
Violin Plots in LUAD II



(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 (H. 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

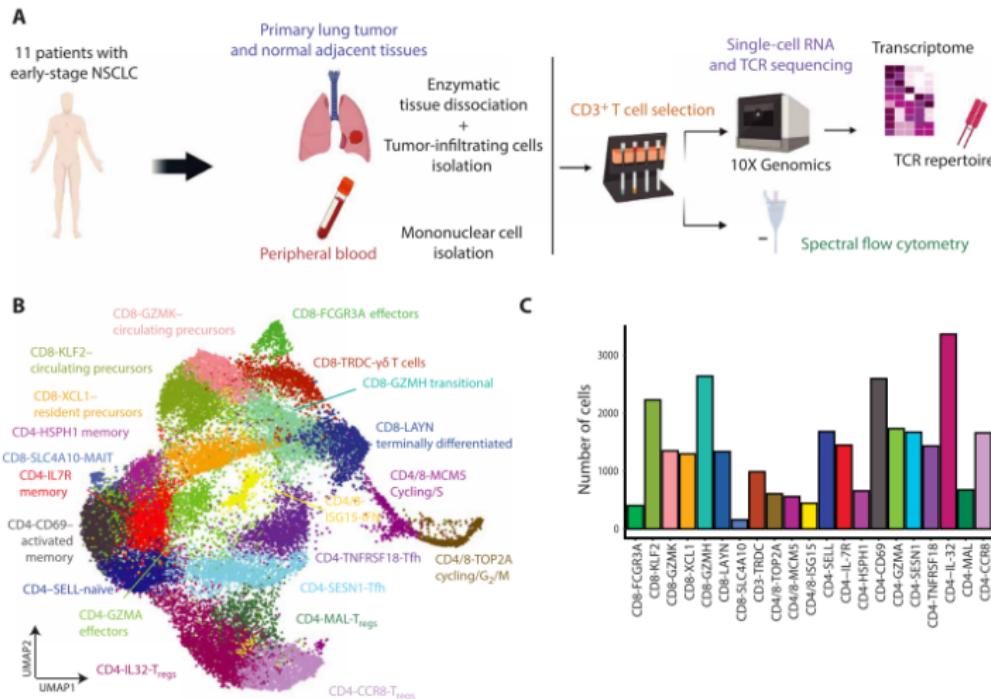


Figure: Characterization of CD3⁺ TILs in NSCLC (Gueguen et al., 2021)

Cluster Plots in LUSC

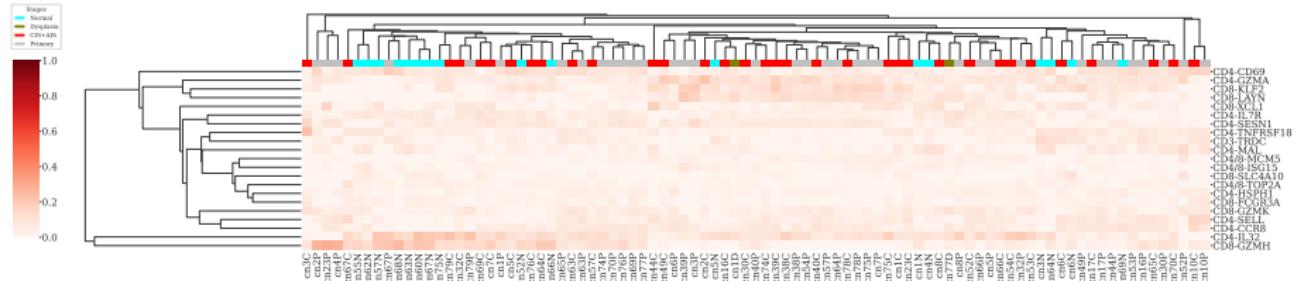
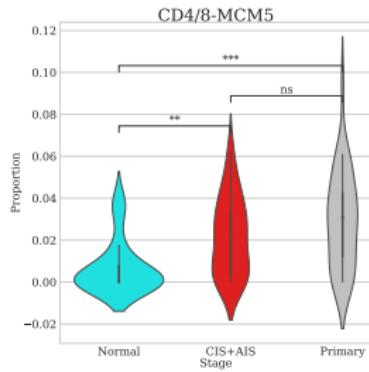
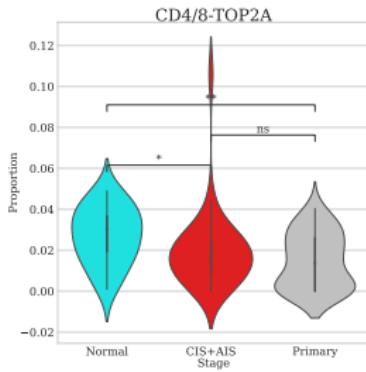


Figure: Cluster Plot in LUAD

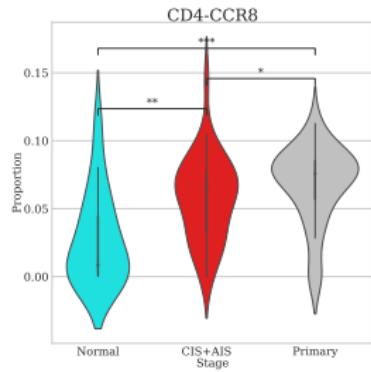
Violin Plots in LUSC I



(a) CD4/8-MCM5



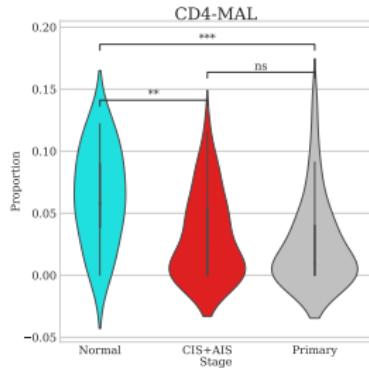
(b) CD4/8-TOP2A



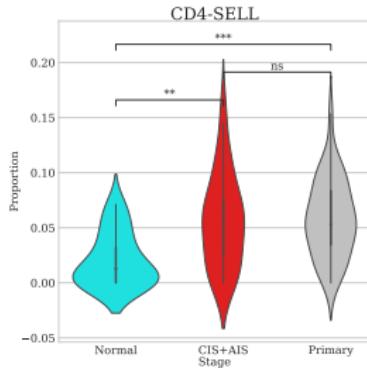
(c) CD4-CCR8

Figure: Violin Plots in LUSC

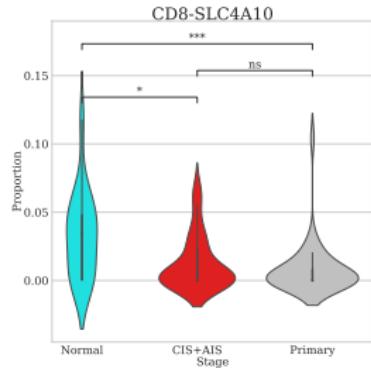
Violin Plots in LUSC II



(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

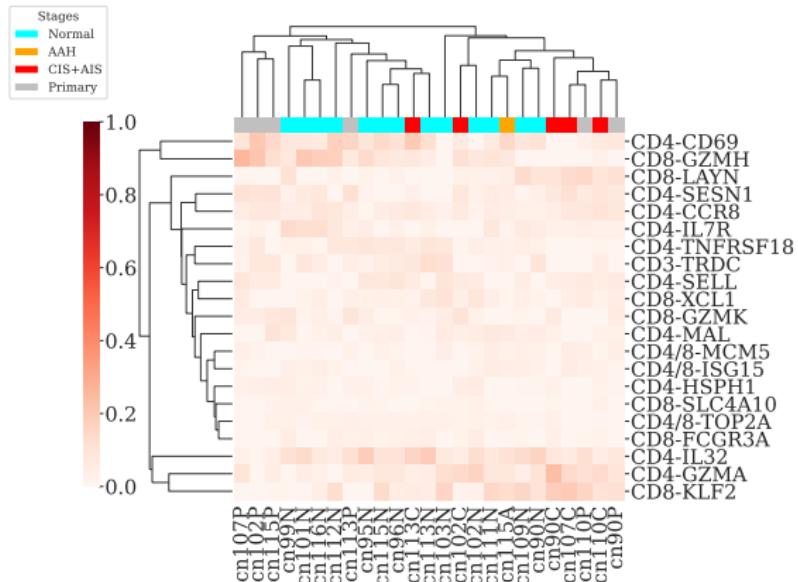
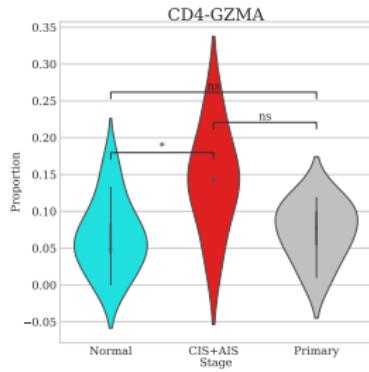
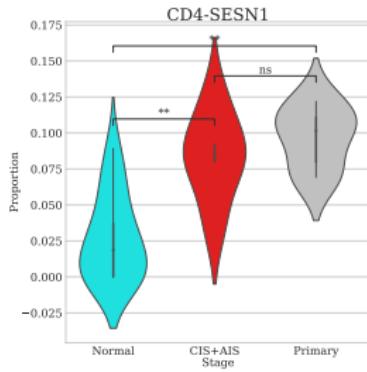


Figure: Cluster Plot in LUAD

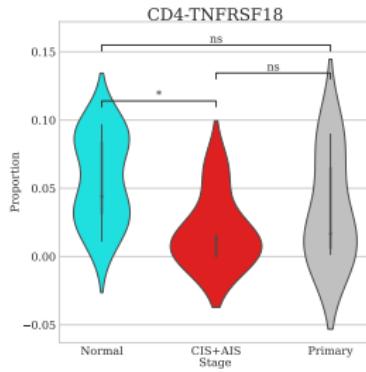
Violin Plots in LUAD



(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?

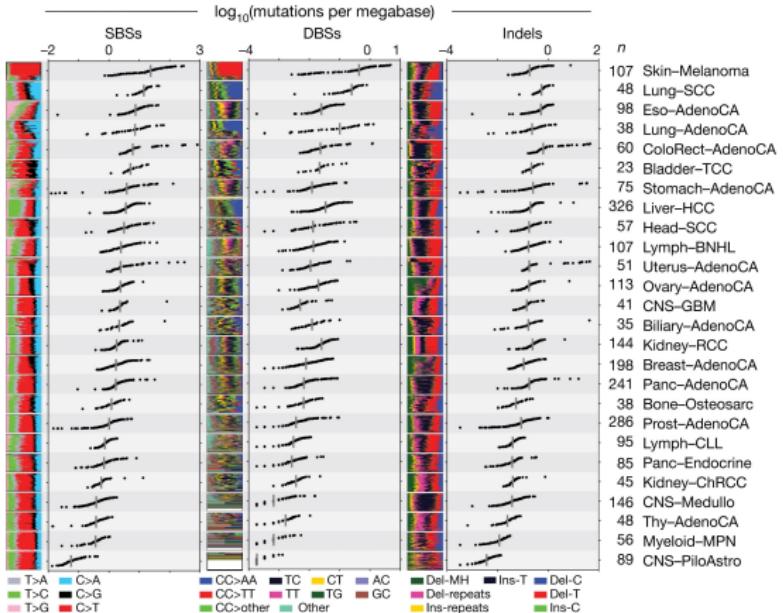


Figure: Mutation Burdens across PCAWG tumor types (Alexandrov et al., 2020)

SigProfiler?

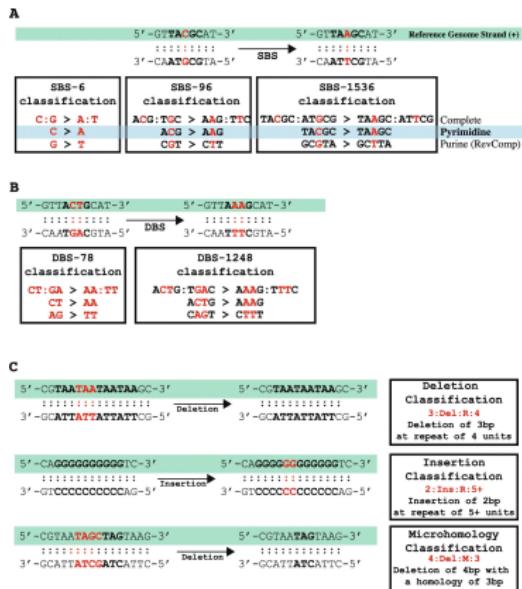


Figure: Classification of mutation signatures by SigProfiler (Bergstrom et al., 2019; Islam et al., 2021; Bergstrom et al., 2020)

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

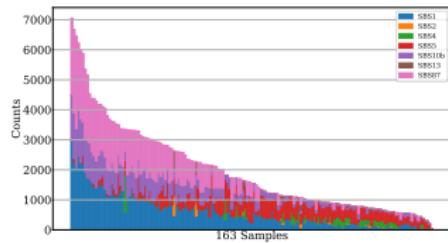
SBS13

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

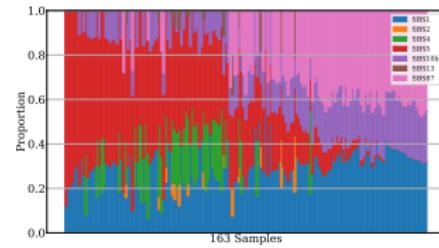
SBS87

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

SBS in LUSC I



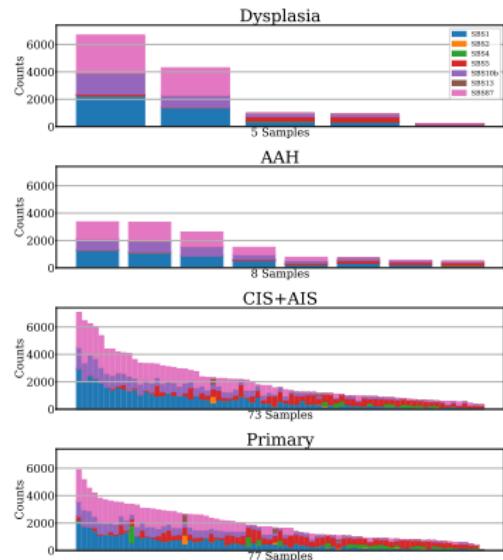
(a) Absolute



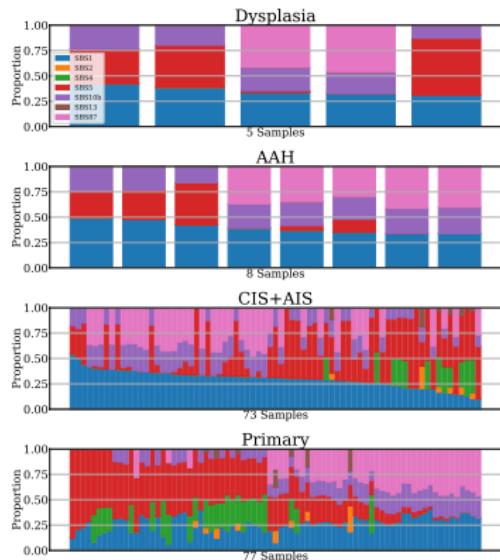
(b) Relative

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

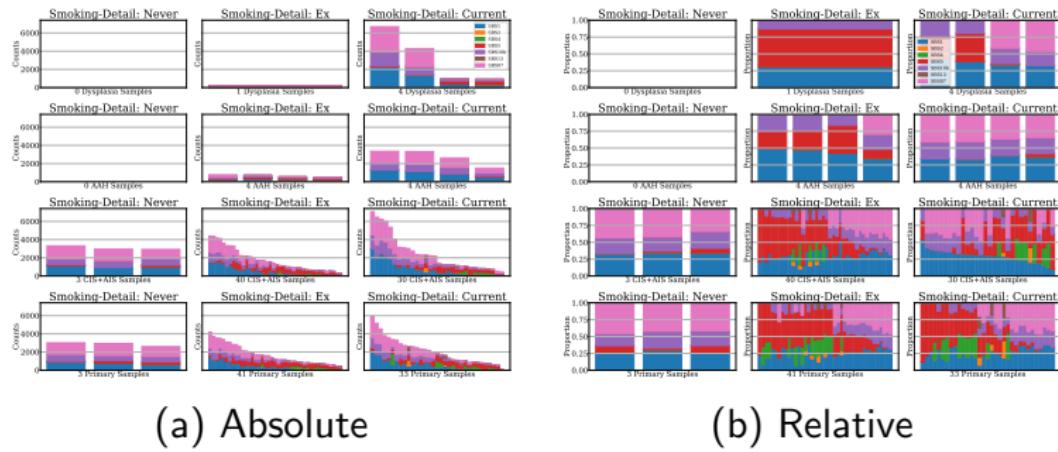
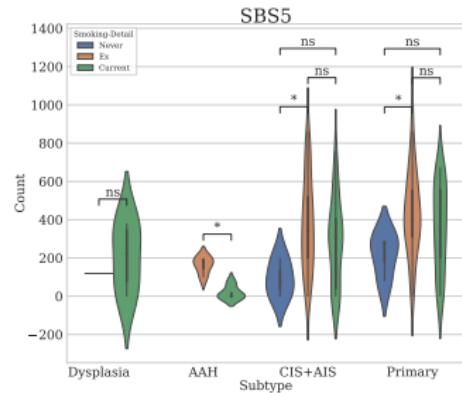
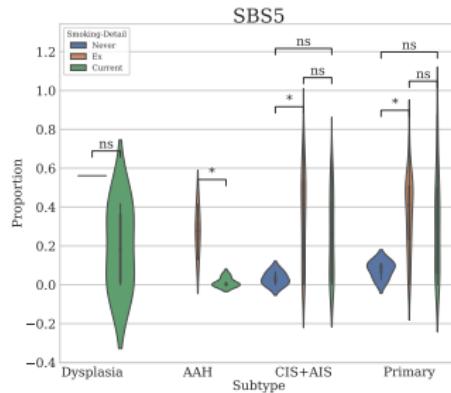


Figure: SBS Bar Plot by Cancer Subtype & Smoking in LUSC

SBS in LUSC with Smoking II



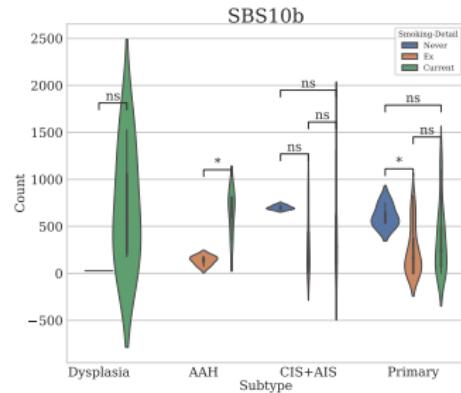
(a) Absolute



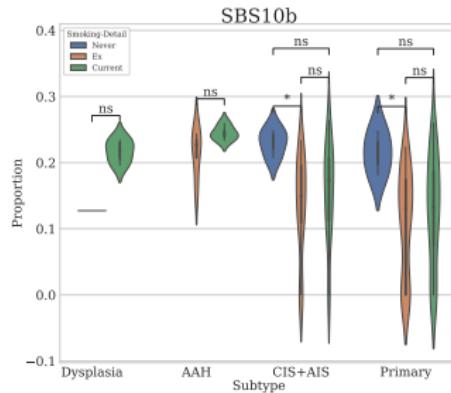
(b) Relative

Figure: SBS5 Signature in LUSC with Smoking

SBS in LUSC with Smoking III



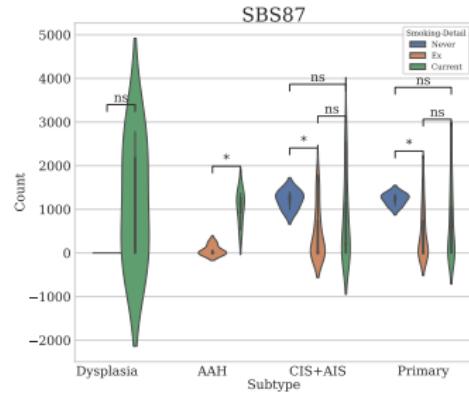
(a) Absolute



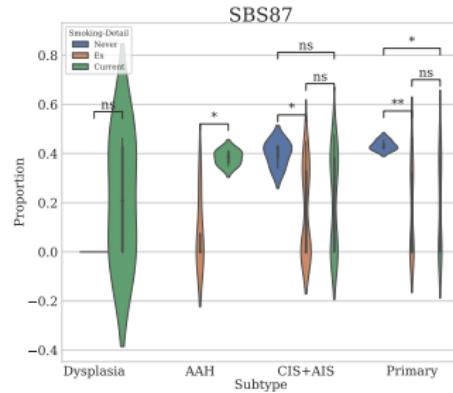
(b) Relative

Figure: SBS10b Signature in LUSC with Smoking

SBS in LUSC with Smoking IV



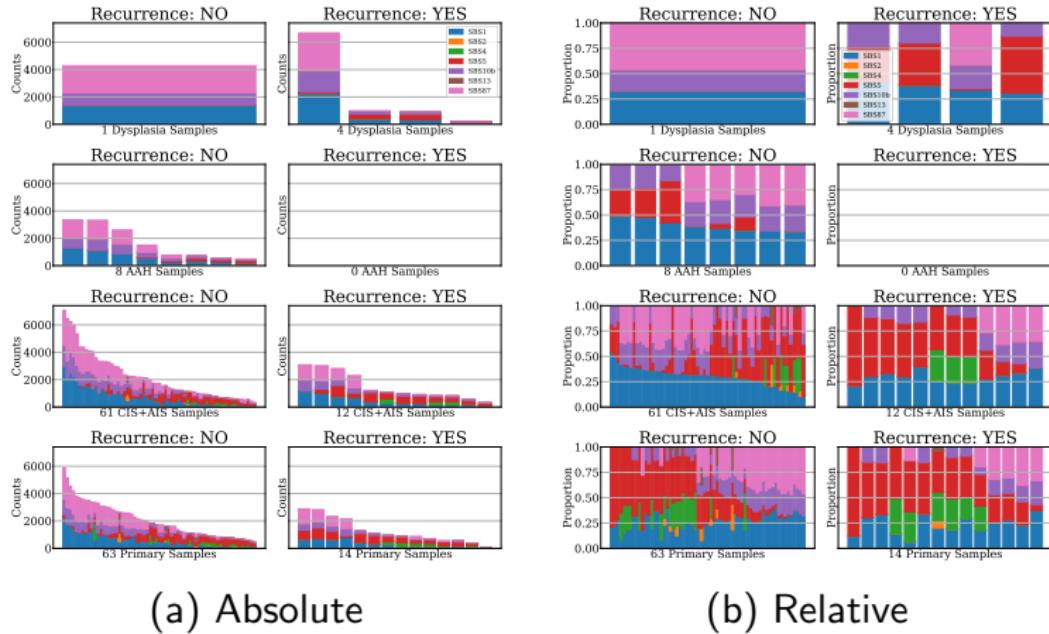
(a) Absolute



(b) Relative

Figure: SBS87 Signature in LUSC with Smoking

SBS in LUSC with Recurrence I

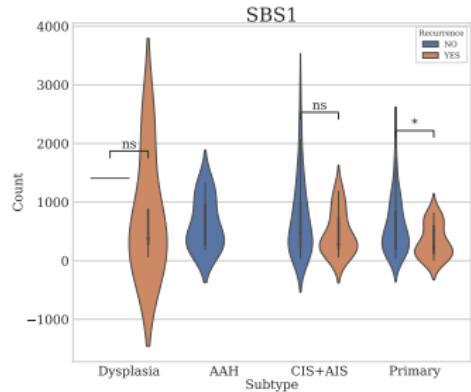


(a) Absolute

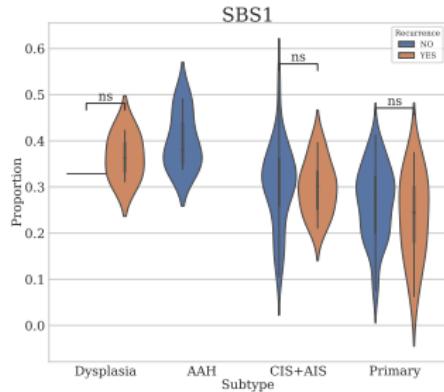
(b) Relative

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

SBS in LUSC with Recurrence II



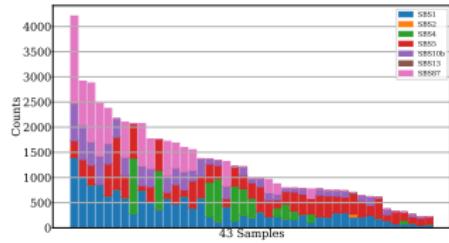
(a) Absolute



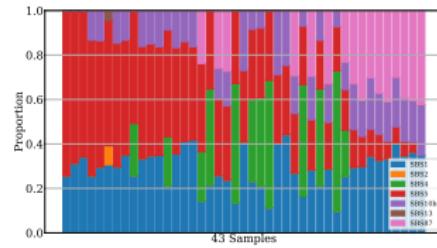
(b) Relative

Figure: SBS1 Signature in LUSC with Recurrence

SBS in LUAD I



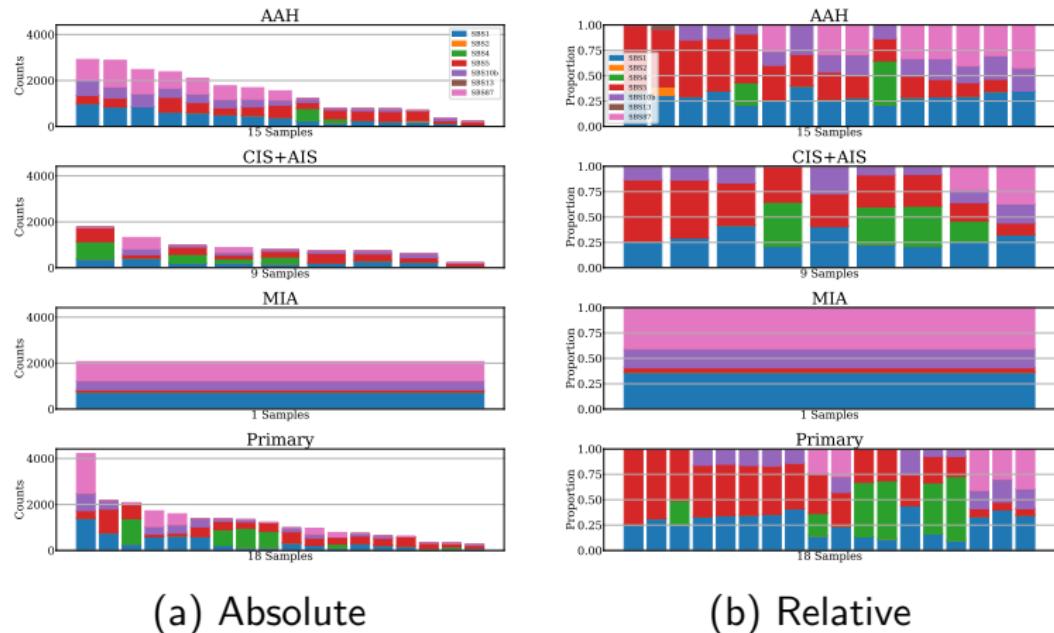
(a) Absolute



(b) Relative

Figure: SBS Bar Plot in LUSC

SBS in LUAD II



(a) Absolute

(b) Relative

Figure: SBS Bar Plot by Cancer Subtype in LUSC

SBS in LUAD with Smoking I

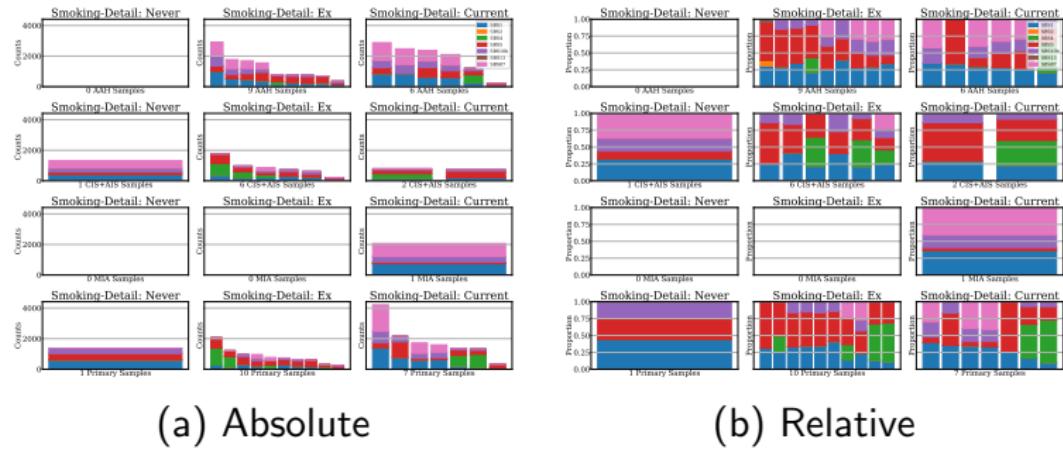
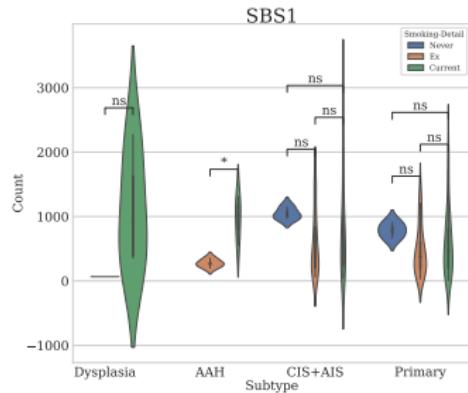
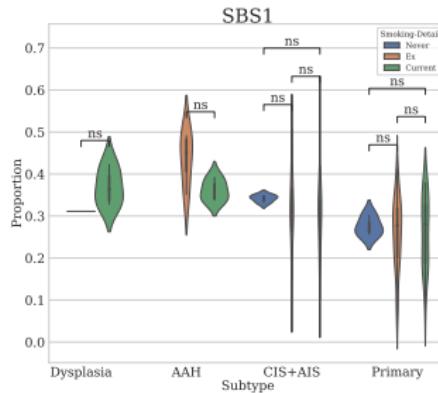


Figure: SBS Bar Plot by Cancer Subtype & Smoking in LUAD

SBS in LUAD with Smoking II



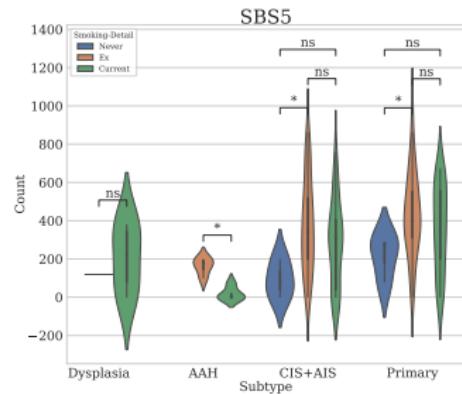
(a) Absolute



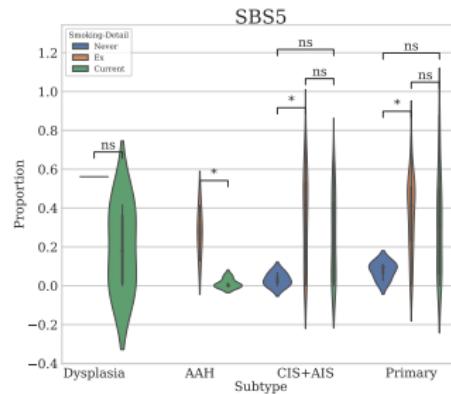
(b) Relative

Figure: SBS1 Signature in LUAD with Smoking

SBS in LUAD with Smoking III



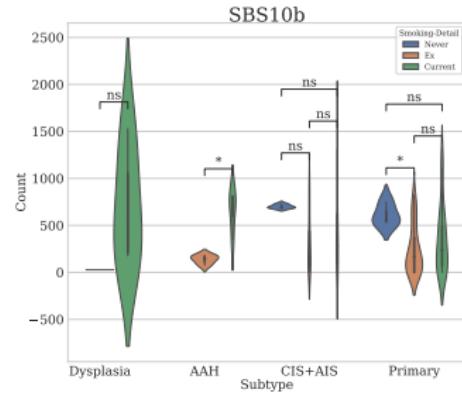
(a) Absolute



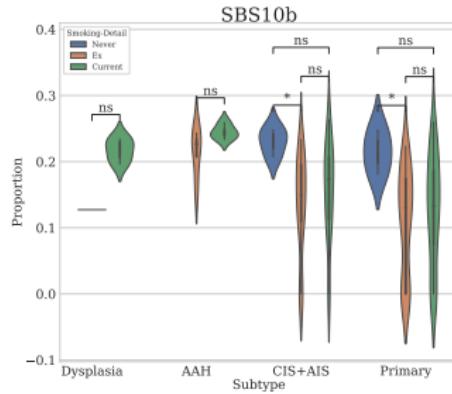
(b) Relative

Figure: SBS5 Signature in LUAD with Smoking

SBS in LUAD with Smoking IV



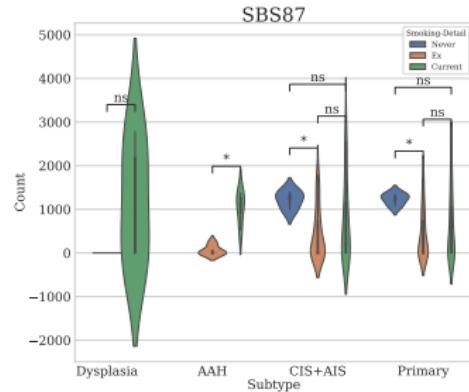
(a) Absolute



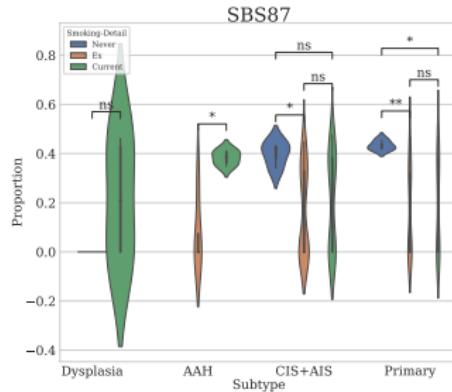
(b) Relative

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

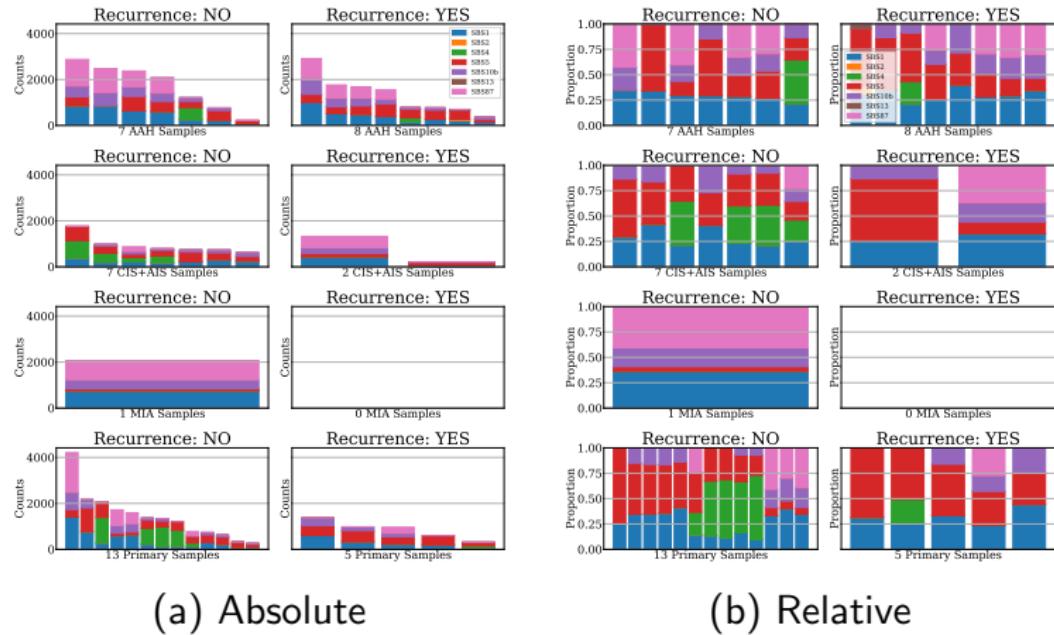
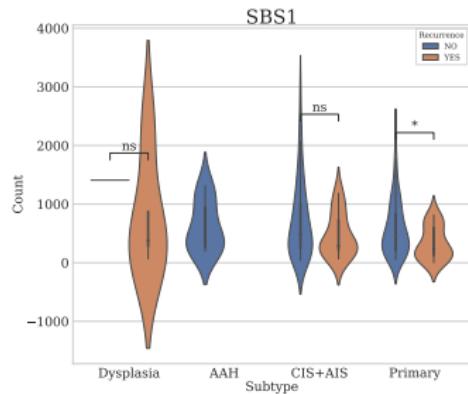
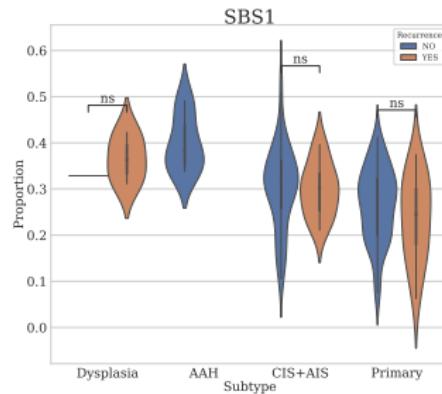


Figure: SBS Bar Plot by Cancer Subtype & Recurrence in LUAD

SBS in LUAD with Recurrence II



(a) Absolute



(b) Relative

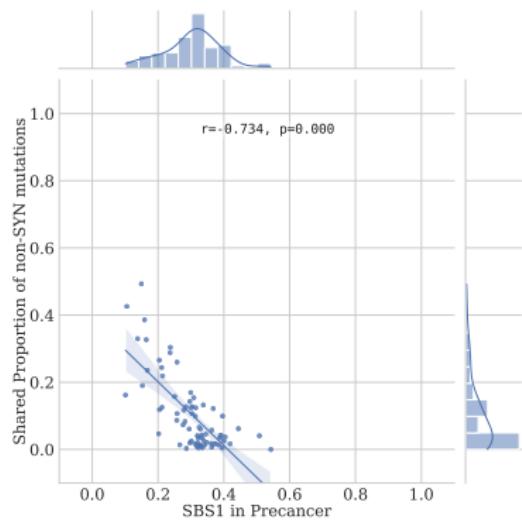
Figure: SBS1 Signature in LUAD with Recurrence

4. Results

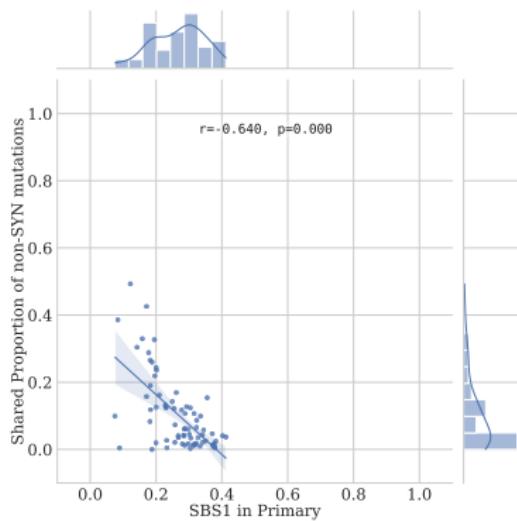
4.9. Discovery of Mutational Signature

4.9.2. SBS with Shared Mutation Proportion in LUSC

SBS1 with Shared Mutation Proportion in LUSC I



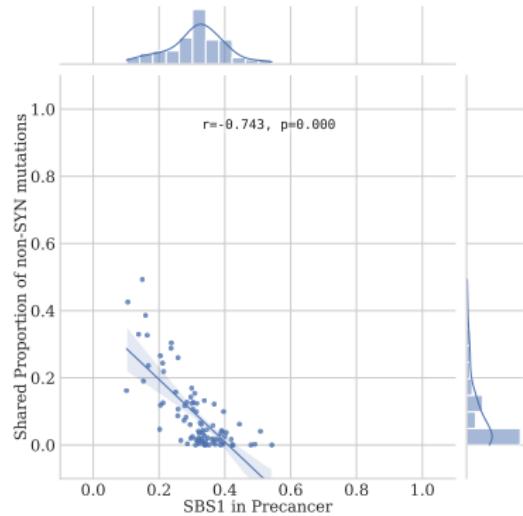
(a) Precancer score



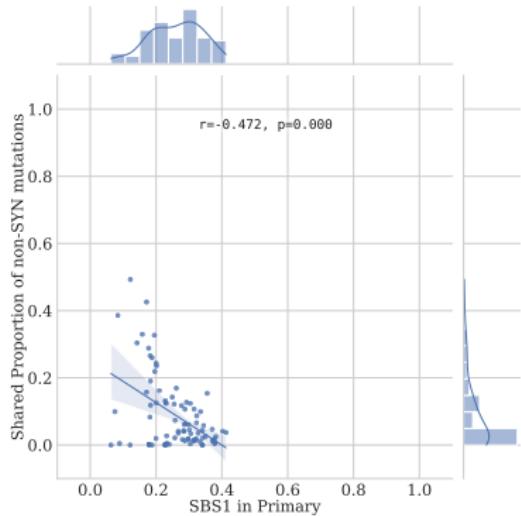
(b) Primary score

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

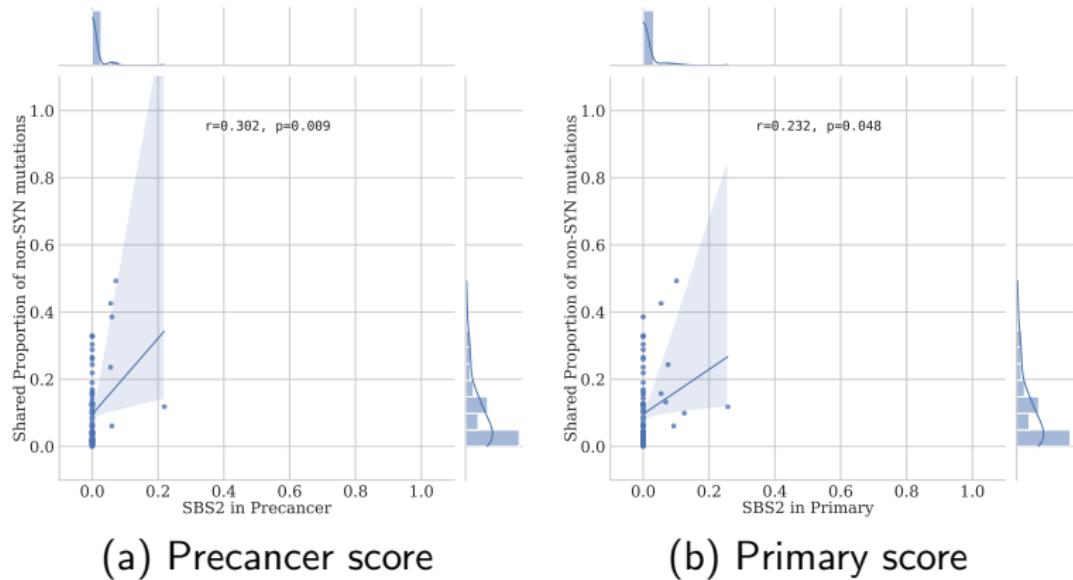
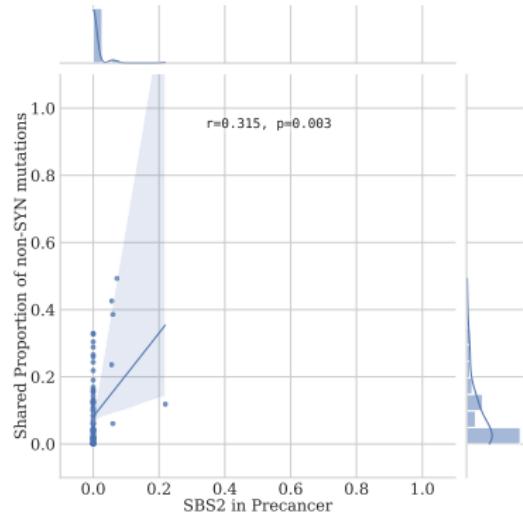
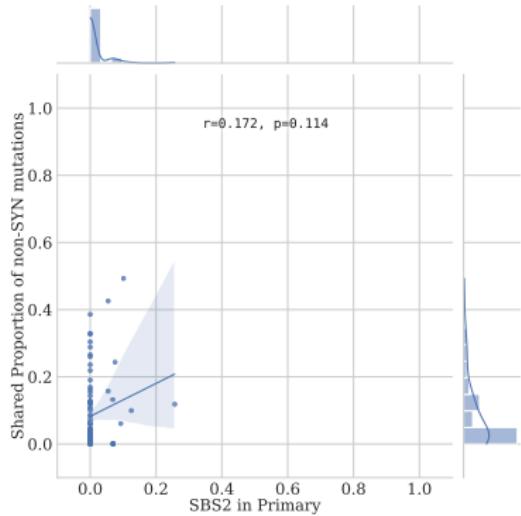


Figure: SBS2 with CIS samples

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

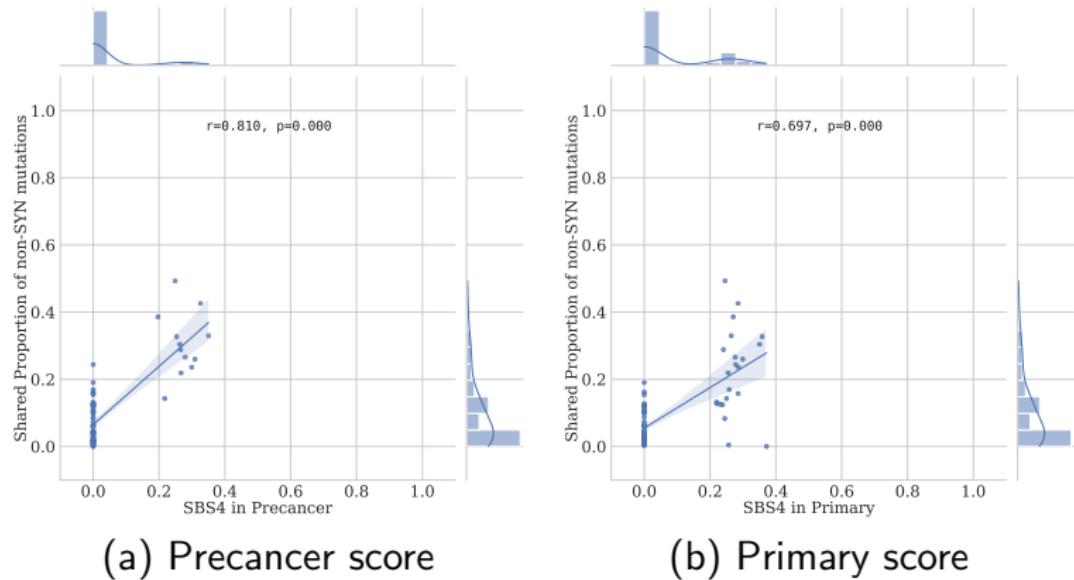
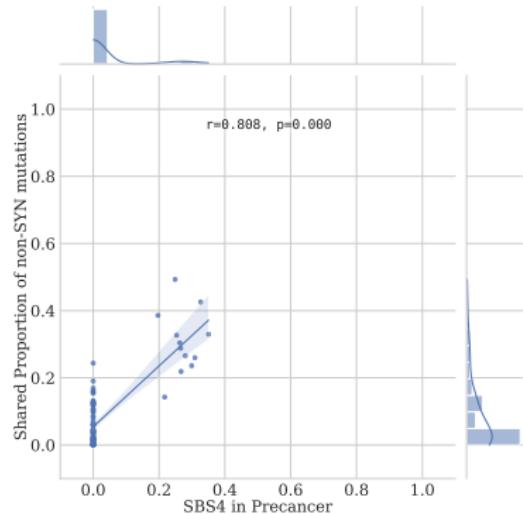
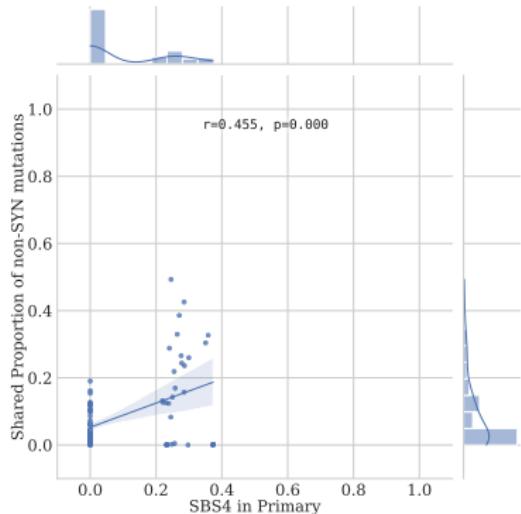


Figure: SBS4 with CIS samples

SBS4 with Shared Mutation Proportion in LUSC II



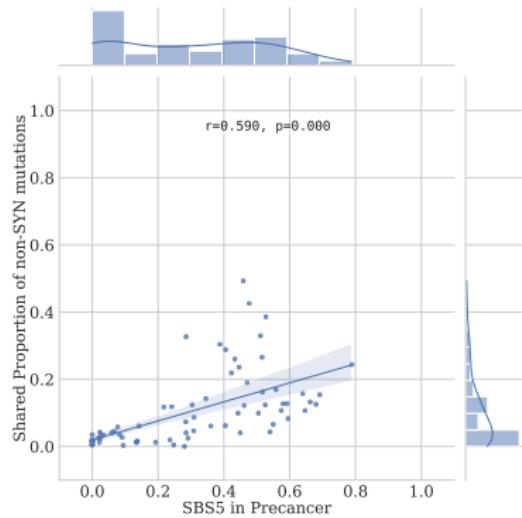
(a) Precancer score



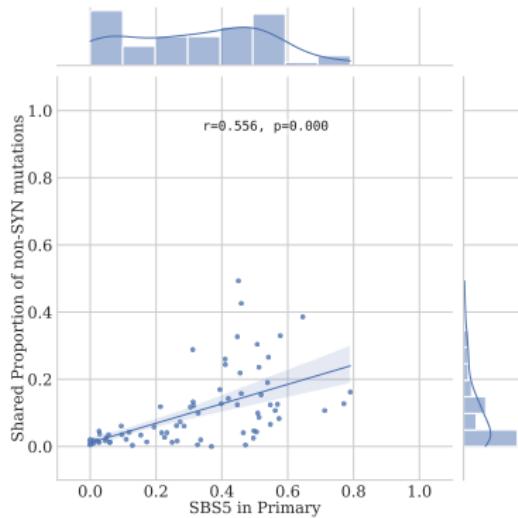
(b) Primary score

Figure: SBS4 with Precancer samples

SBS5 with Shared Mutation Proportion in LUSC I



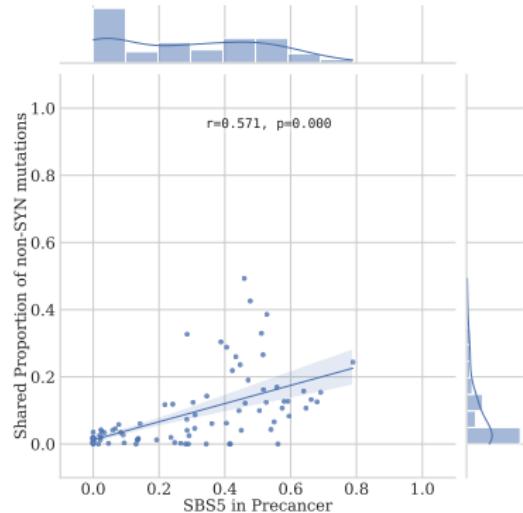
(a) Precancer score



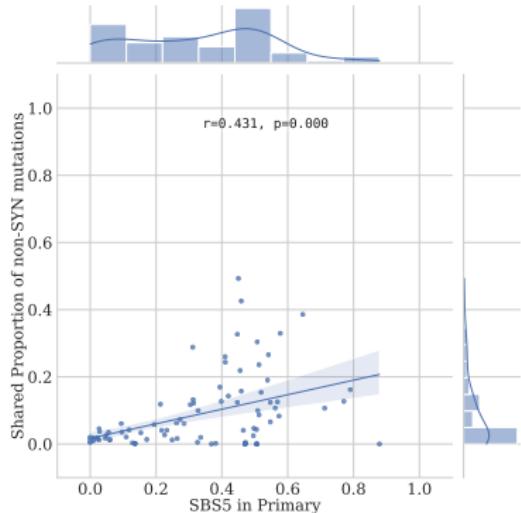
(b) Primary score

Figure: SBS5 with CIS samples

SBS5 with Shared Mutation Proportion in LUSC II



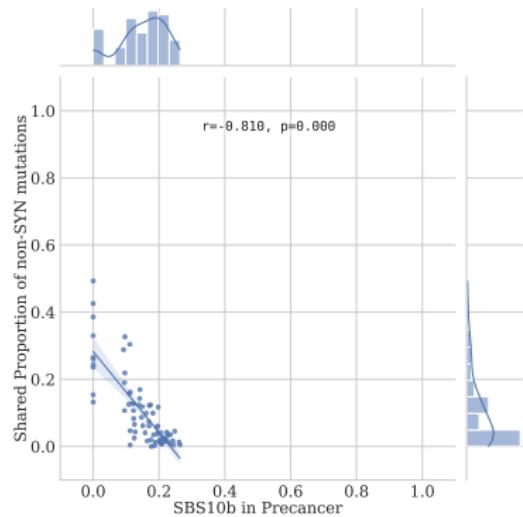
(a) Precancer score



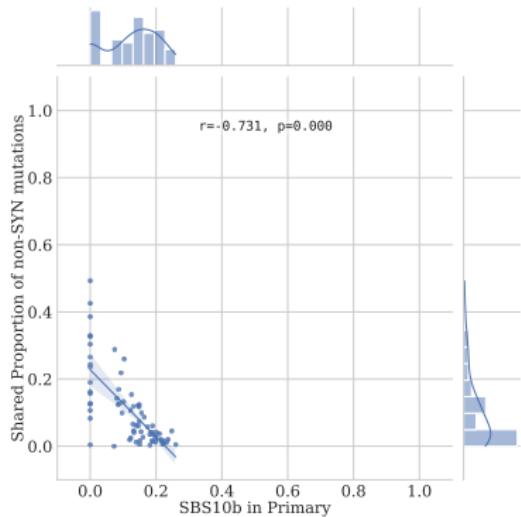
(b) Primary score

Figure: SBS5 with Precancer samples

SBS10b with Shared Mutation Proportion in LUSC I



(a) Precancer score



(b) Primary score

Figure: SBS10b with CIS samples

SBS10b with Shared Mutation Proportion in LUSC II

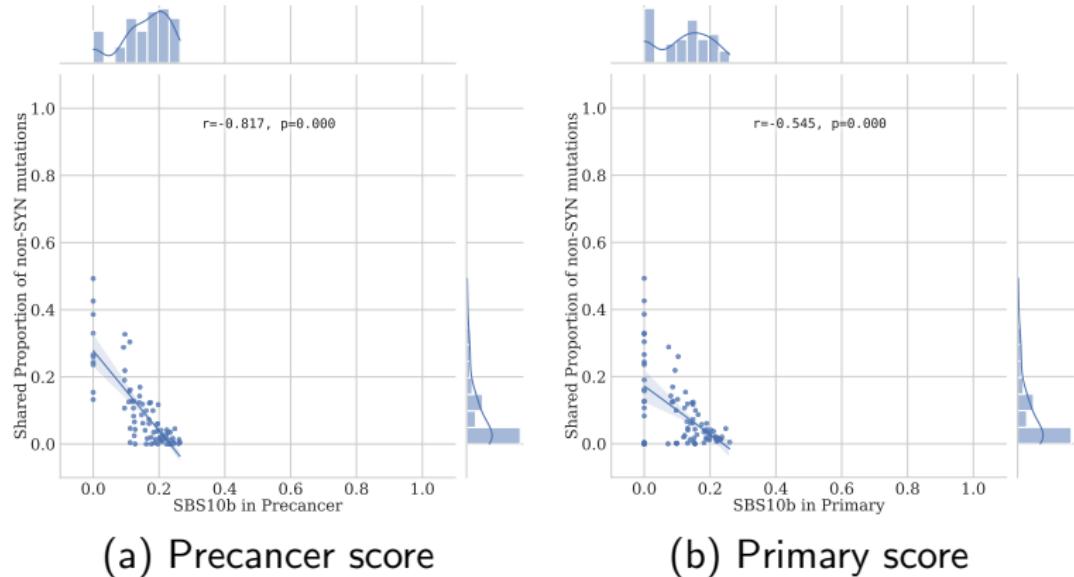
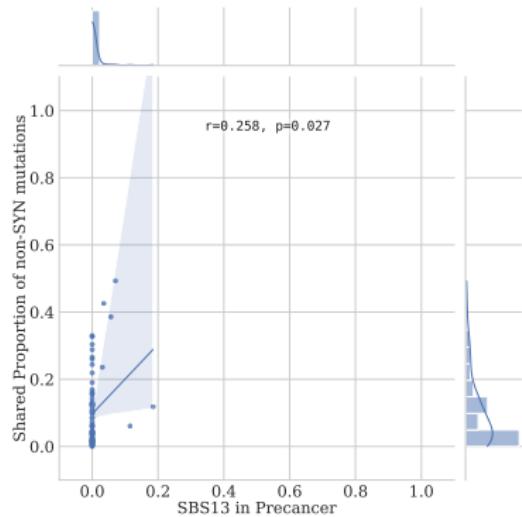
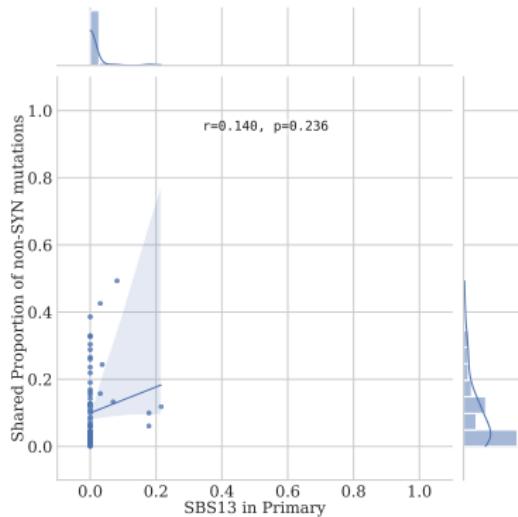


Figure: SBS10b with Precancer samples

SBS13 with Shared Mutation Proportion in LUSC I



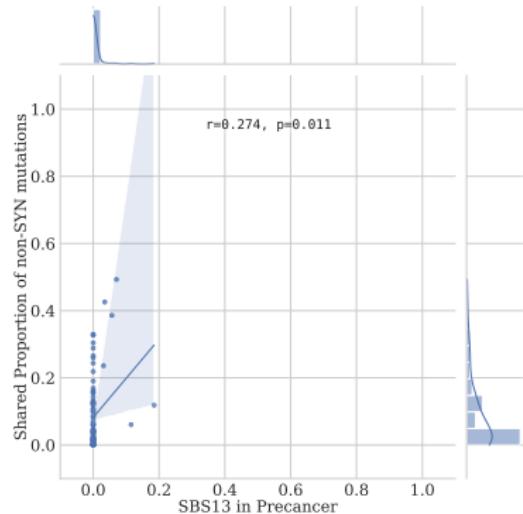
(a) Precancer score



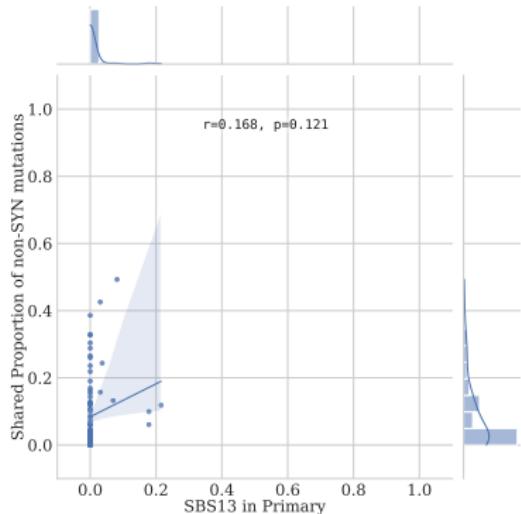
(b) Primary score

Figure: SBS13 with CIS samples

SBS13 with Shared Mutation Proportion in LUSC II



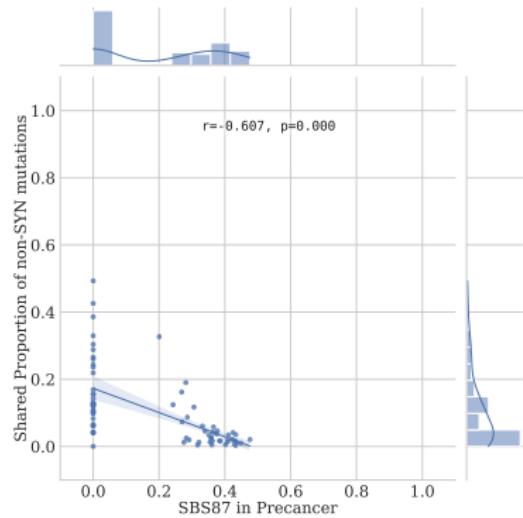
(a) Precancer score



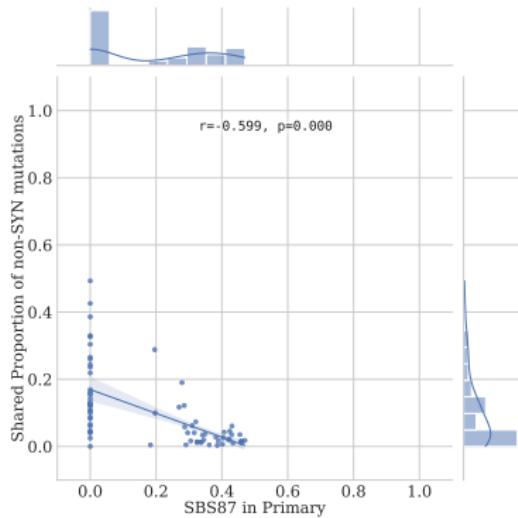
(b) Primary score

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

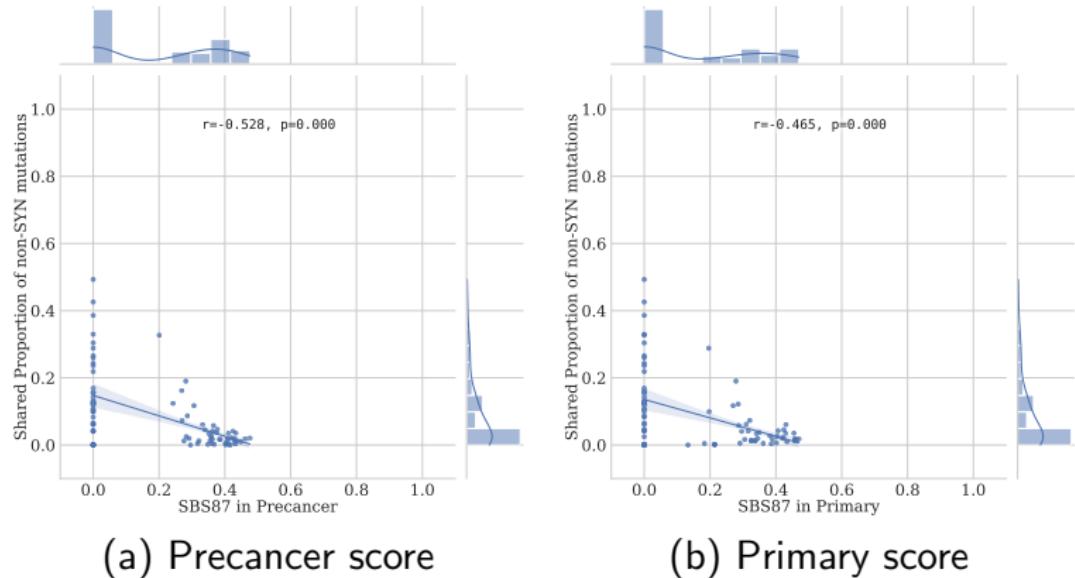


Figure: SBS87 with Precancer samples

4. Results

4.9. Discovery of Mutational Signature

4.9.3. SBS with Clinical Values in LUSC

SBS5 with Volume Doubling Time in LUSC

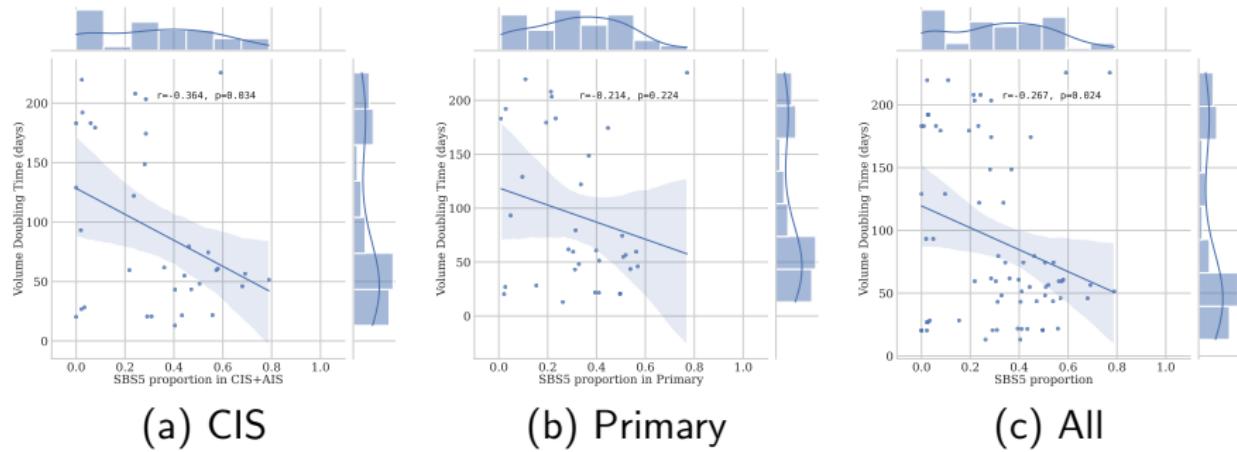
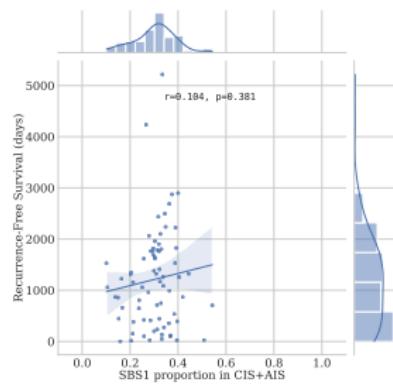
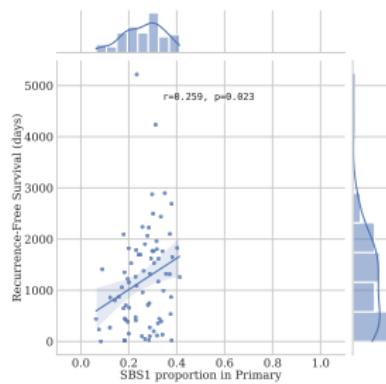


Figure: SBS5 with VDT in LUSC

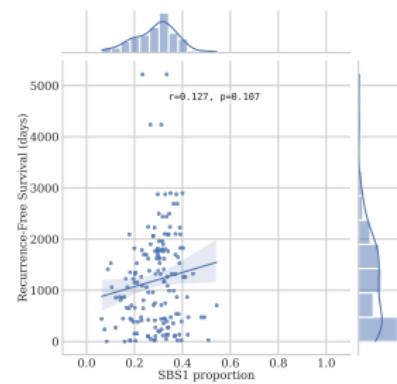
SBS1 with Recurrence-Free Survival in LUSC



(a) CIS



(b) Primary



(c) All

Figure: SBS1 with RFS in LUSC

SBS5 with Recurrence-Free Survival in LUSC

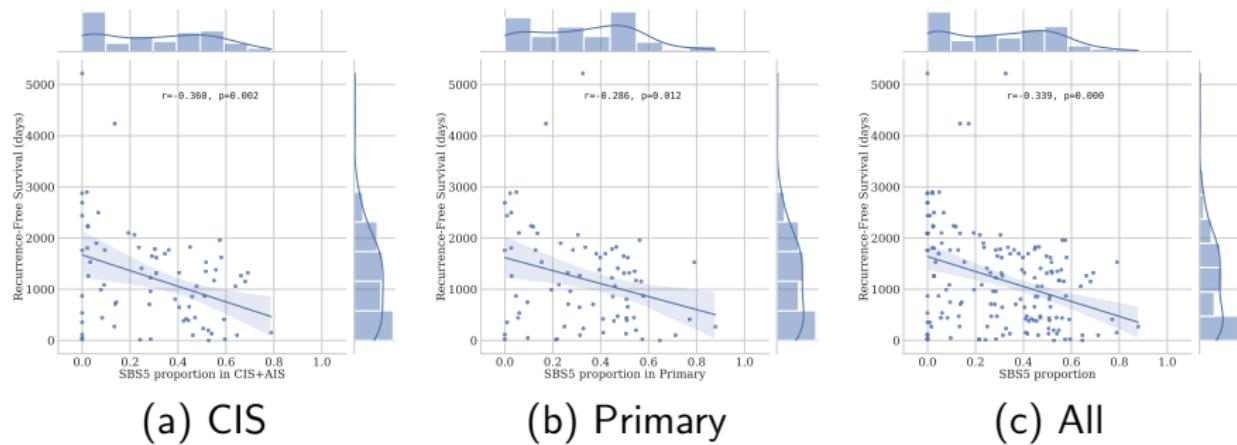
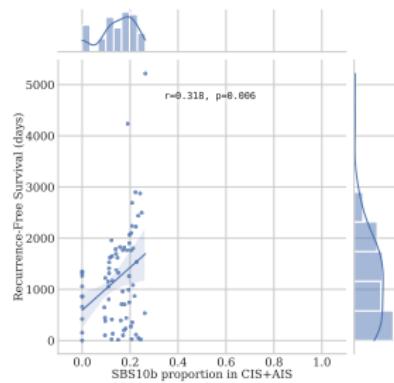
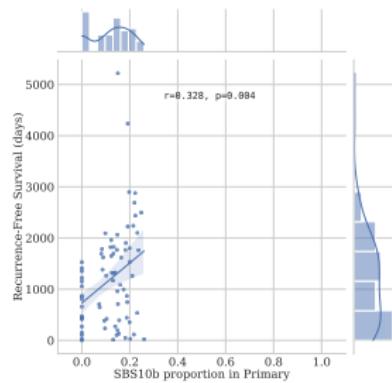


Figure: SBS5 with RFS in LUSC

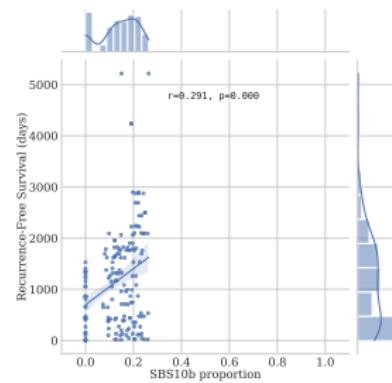
SBS10b with Recurrence-Free Survival in LUSC



(a) CIS



(b) Primary



(c) All

Figure: SBS10b with RFS in LUSC

SBS87 with Recurrence-Free Survival in LUSC

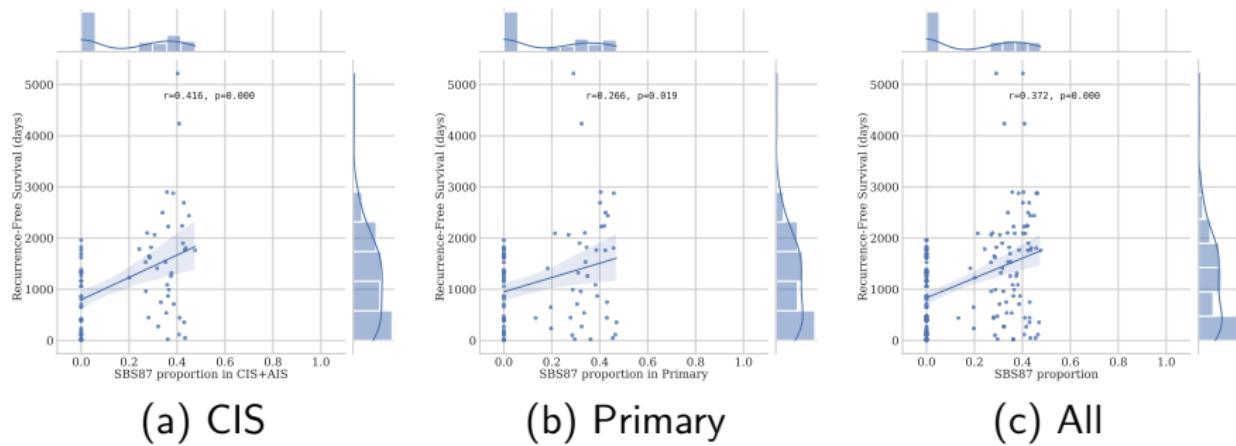


Figure: SBS87 with RFS in LUSC

SBS1 with Overall Survival in LUSC

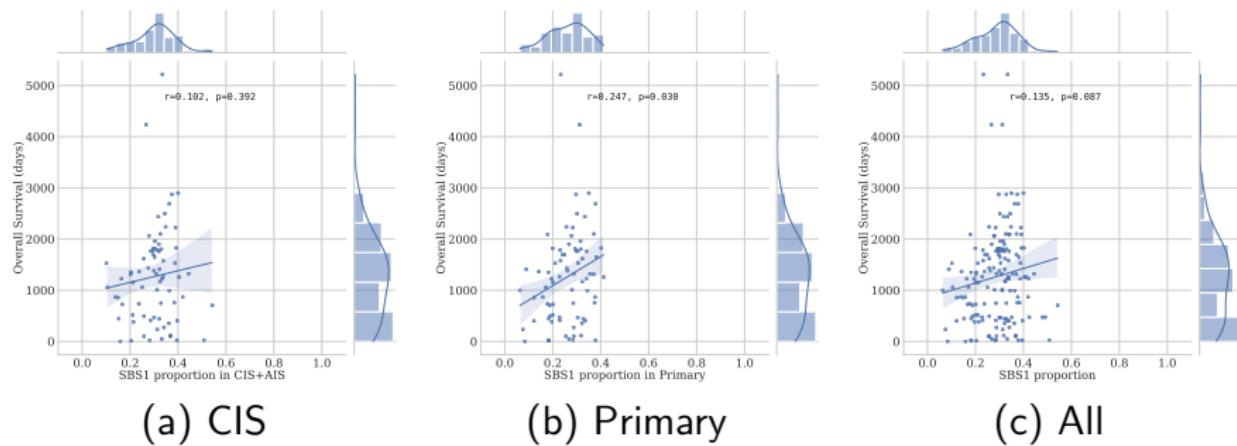


Figure: SBS1 with OS in LUSC

SBS5 with Overall Survival in LUSC

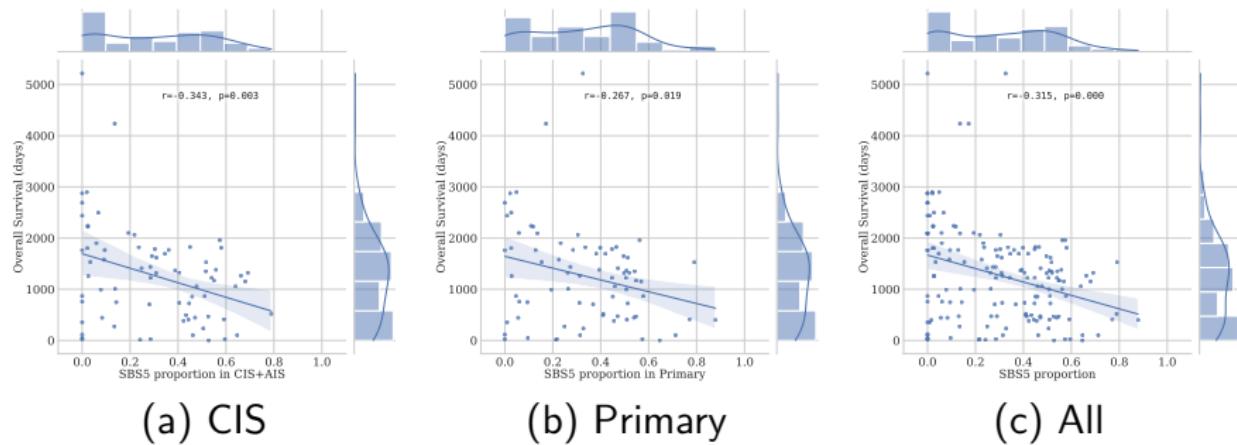


Figure: SBS5 with OS in LUSC

SBS10b with Overall Survival in LUSC

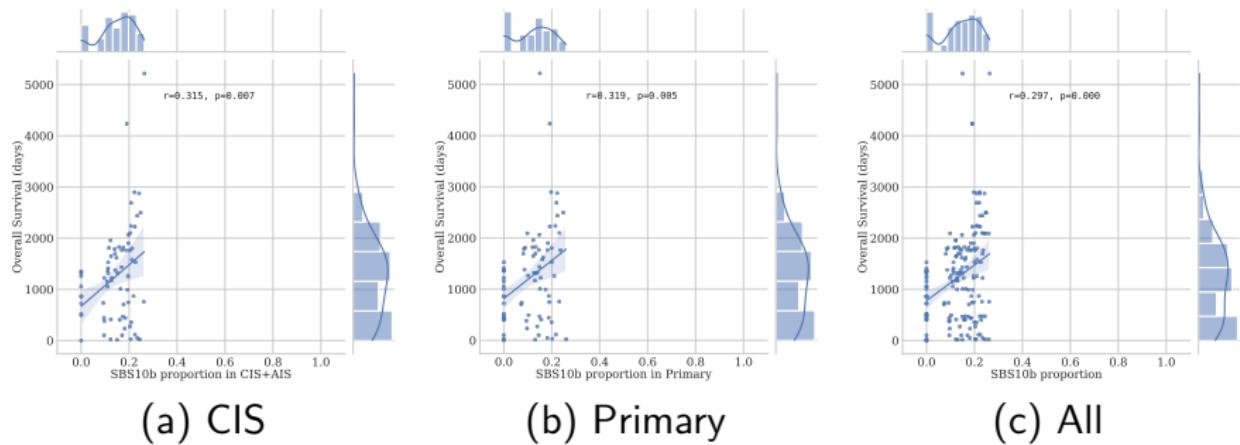


Figure: SBS10b with CIS samples

SBS87 with Overall Survival in LUSC

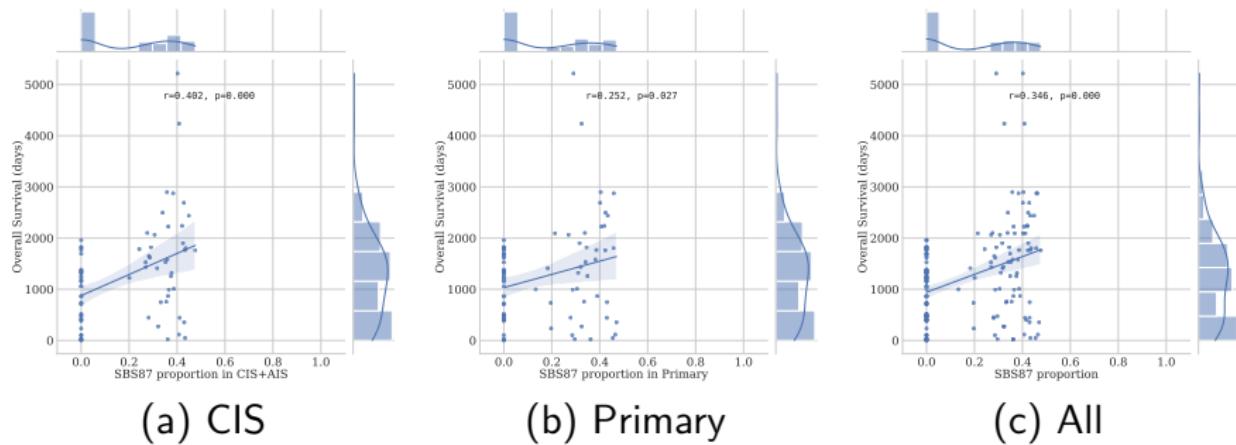


Figure: SBS87 with OS in LUSC

4. Results

4.9. Discovery of Mutational Signature

4.9.4. SBS with Clinical Values in LUAD

SBS5 with Overall Survival in LUAD

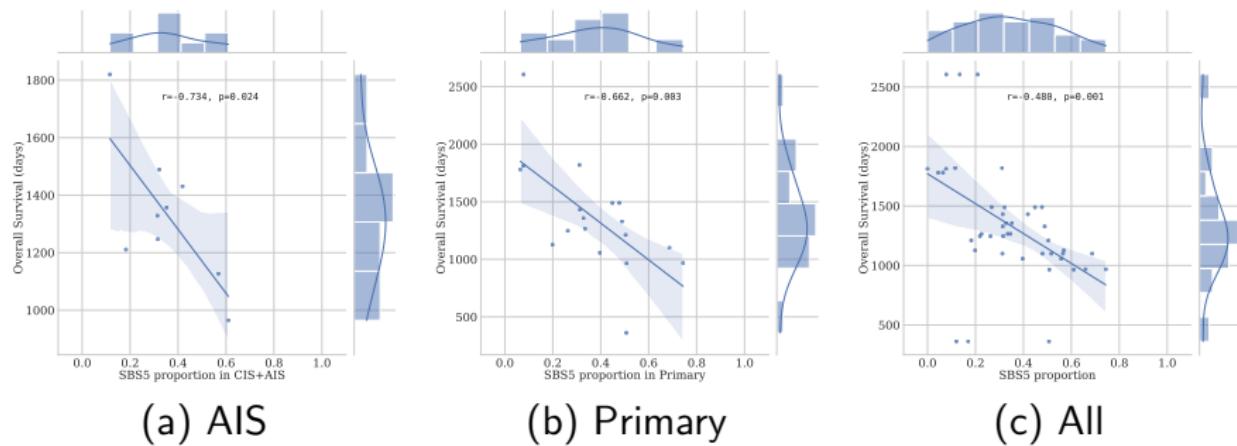


Figure: SBS5 with OS in LUAD

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

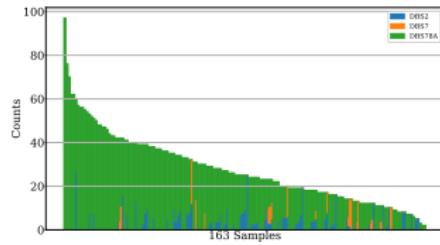
DBS7

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

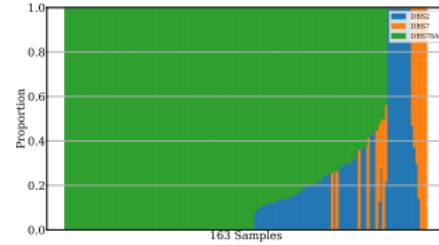
DBS78A

- Unknown

DBS in LUSC I



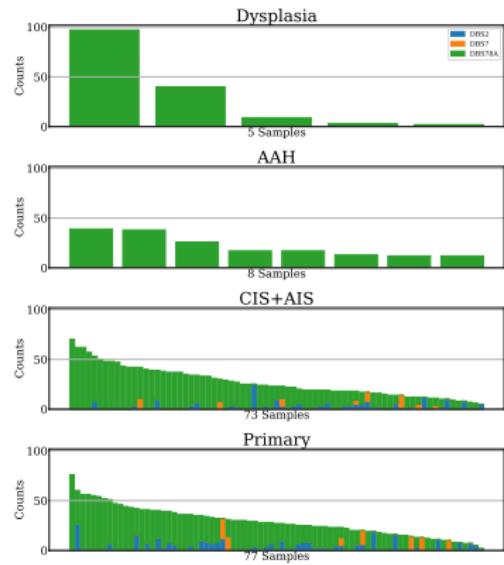
(a) Absolute



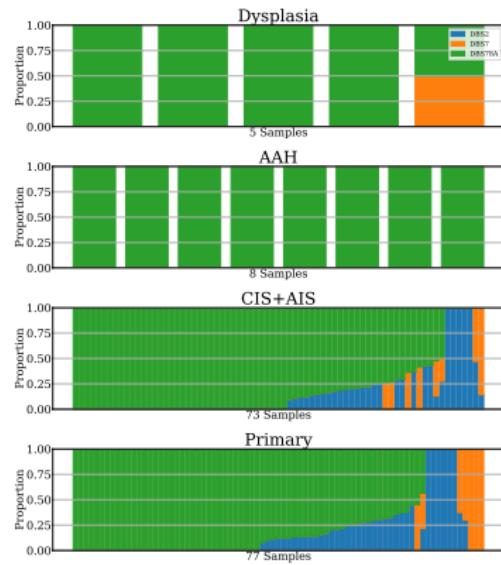
(b) Relative

Figure: DBS Bar Plot in LUSC

DBS in LUSC II



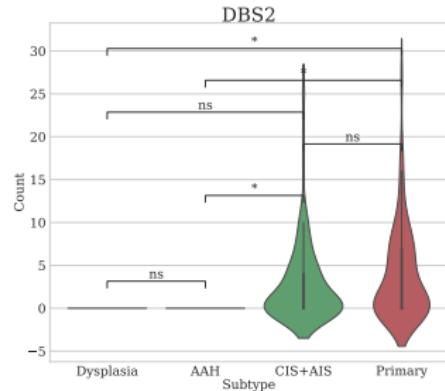
(a) Absolute



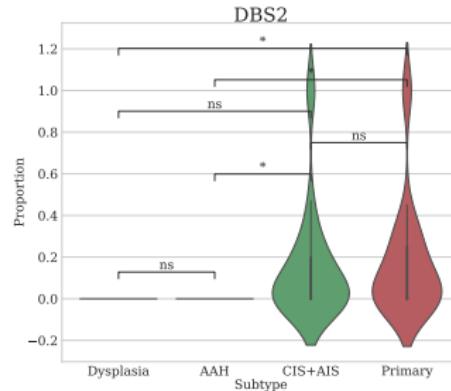
(b) Relative

Figure: DBS Bar Plot by Cancer Subtype in LUSC

DBS in LUSC III



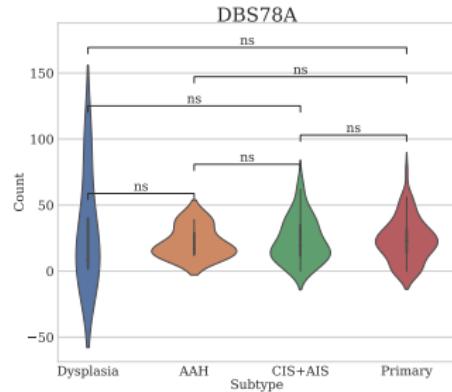
(a) Absolute



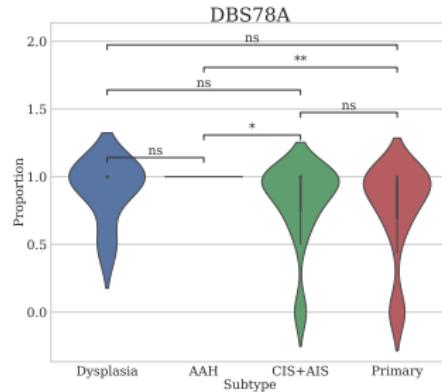
(b) Relative

Figure: DBS2 Signature in LUSC

DBS in LUSC IV



(a) Absolute



(b) Relative

Figure: DBS78A Signature in LUSC

DBS in LUSC with Smoking I

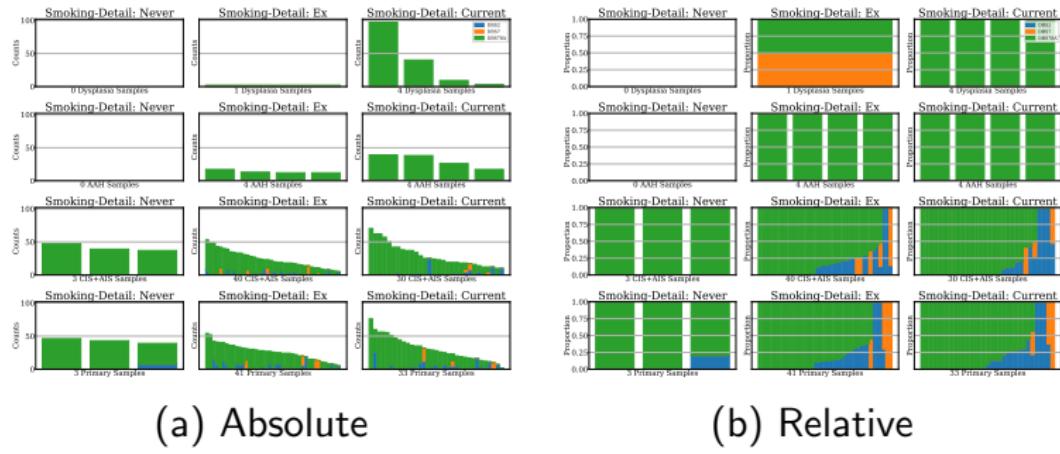
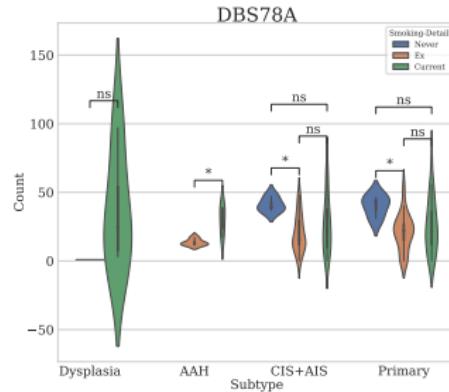
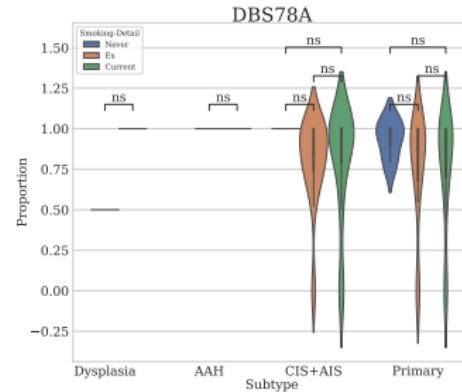


Figure: DBS Bar Plot by Cancer Subtype & Smoking in LUSC

DBS in LUSC with Smoking II



(a) Absolute



(b) Relative

Figure: DBS78A Signature in LUSC with Smoking

DBS in LUSC with Recurrence

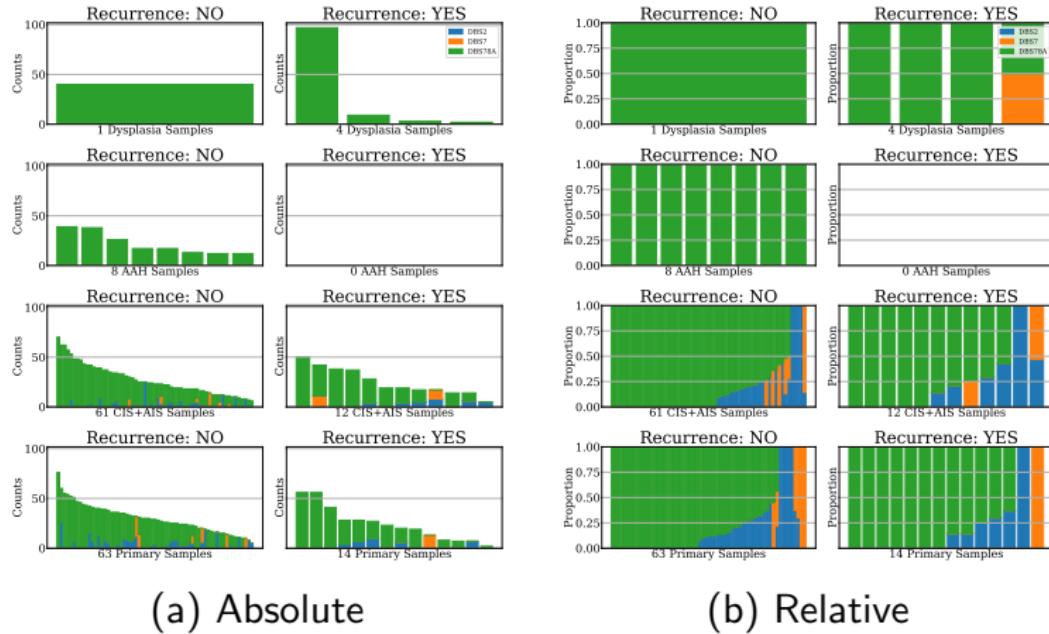
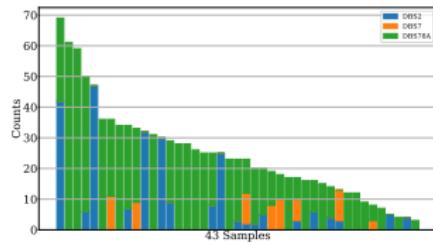
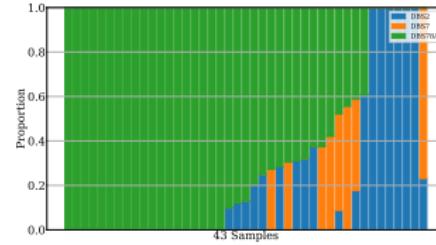


Figure: DBS Bar Plot by Cancer Subtype & Recurrence in LUSC

DBS in LUAD I



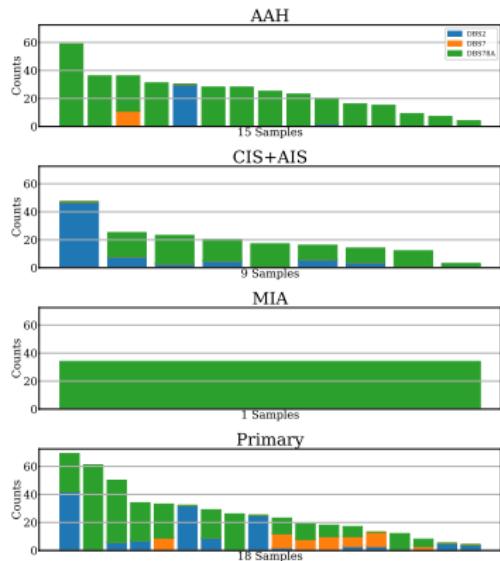
(a) Absolute



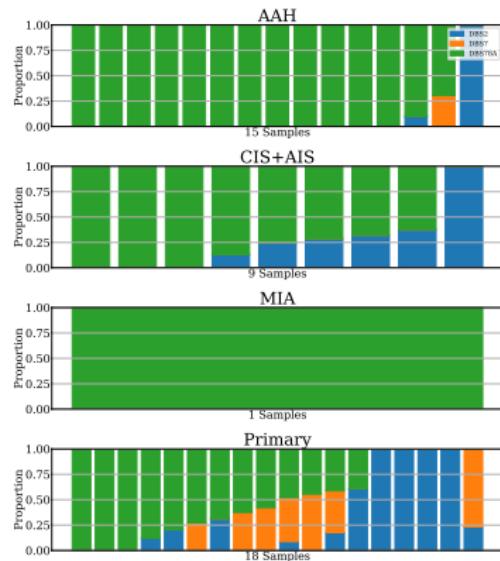
(b) Relative

Figure: DBS Bar Plot in LUAD

DBS in LUAD II



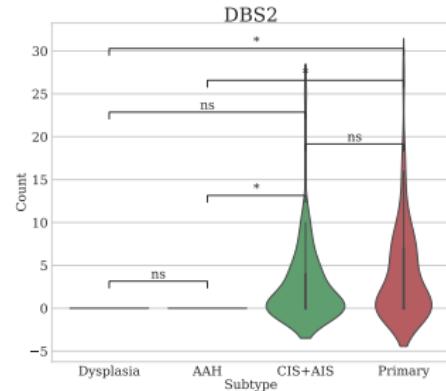
(a) Absolute



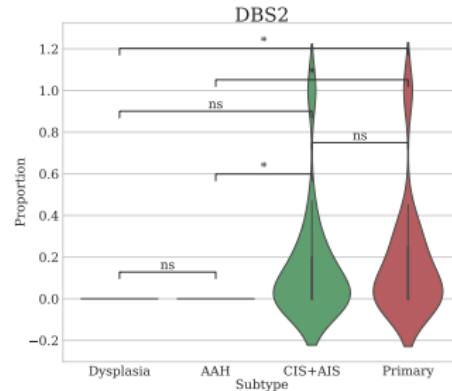
(b) Relative

Figure: DBS Bar Plot by Cancer Subtype in LUAD

DBS in LUAD III



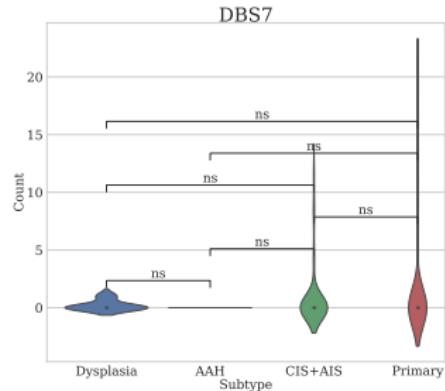
(a) Absolute



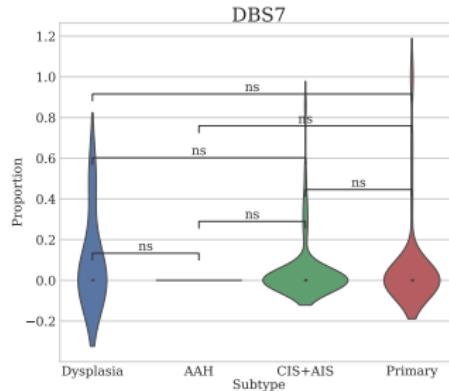
(b) Relative

Figure: DBS2 Signature in LUSC

DBS in LUAD IV



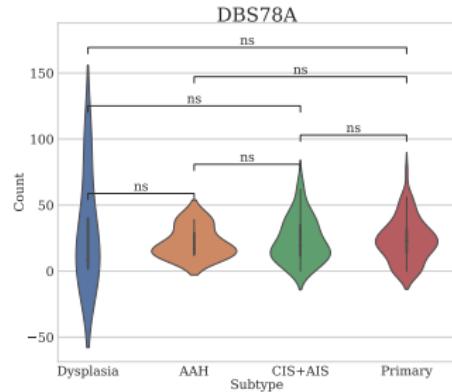
(a) Absolute



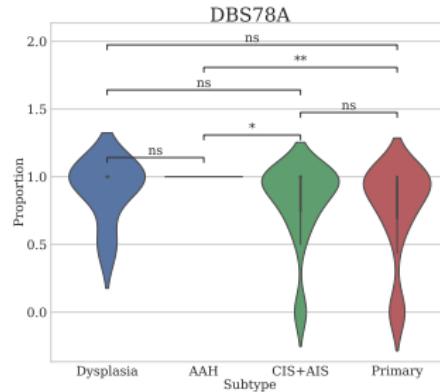
(b) Relative

Figure: DBS7 Signature in LUSC

DBS in LUAD V



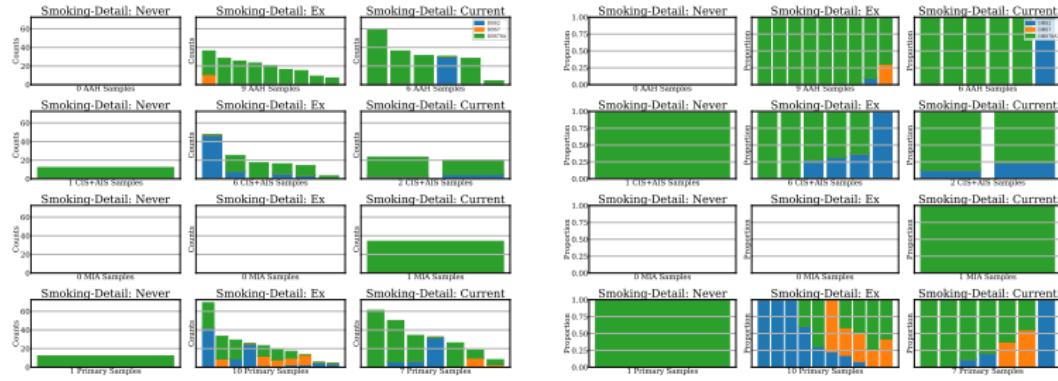
(a) Absolute



(b) Relative

Figure: DBS78A Signature in LUSC

DBS in LUAD with Smoking I

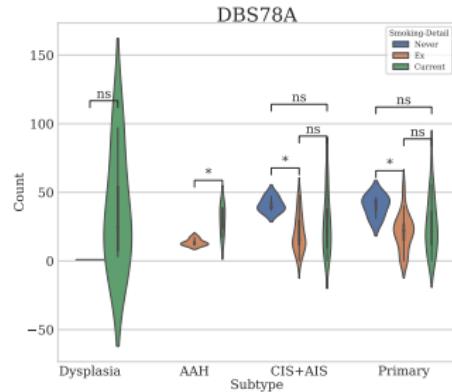


(a) Absolute

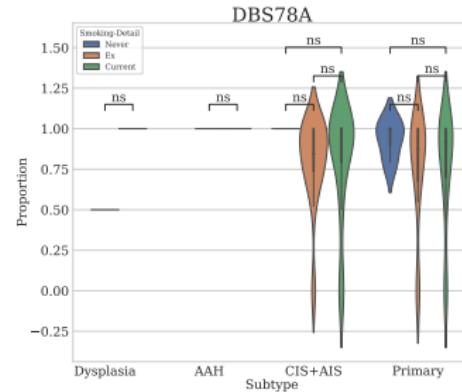
(b) Relative

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

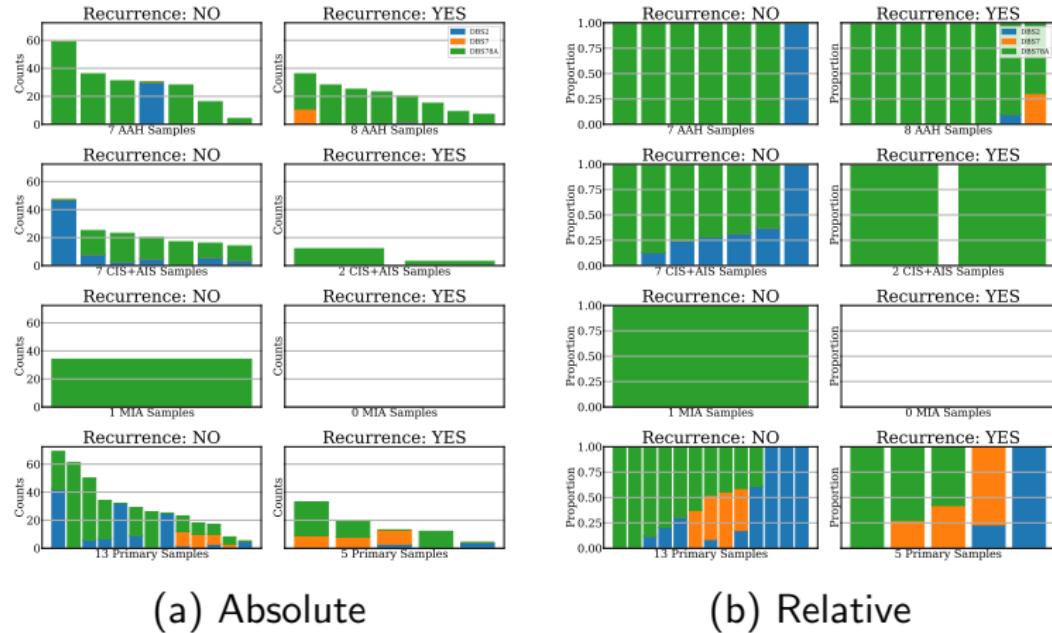


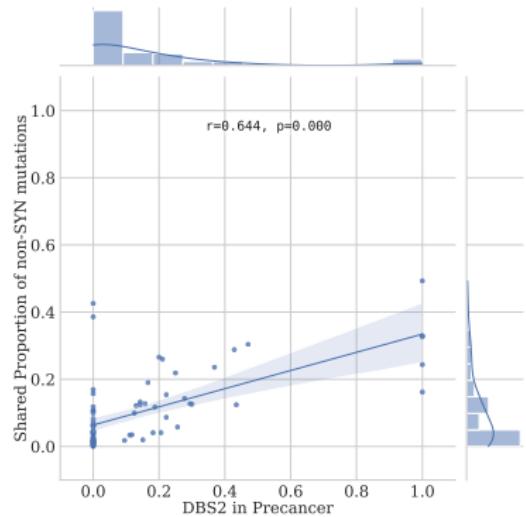
Figure: DBS Bar Plot by Cancer Subtype & Recurrence in LUAD

4. Results

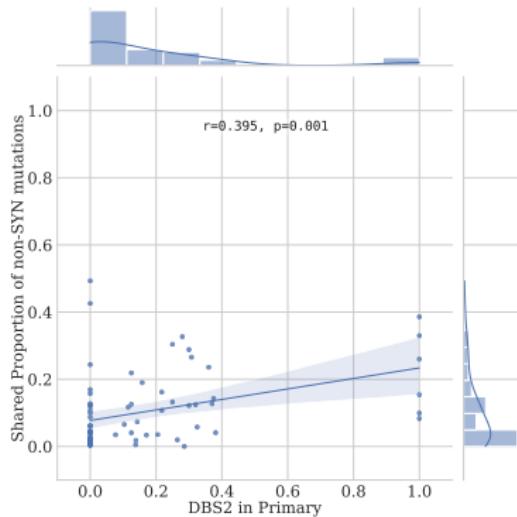
4.9. Discovery of Mutational Signature

4.9.6. DBS with Shared mutation Proportion in LUSC

DBS2 with Shared Mutation Proportion in LUSC I



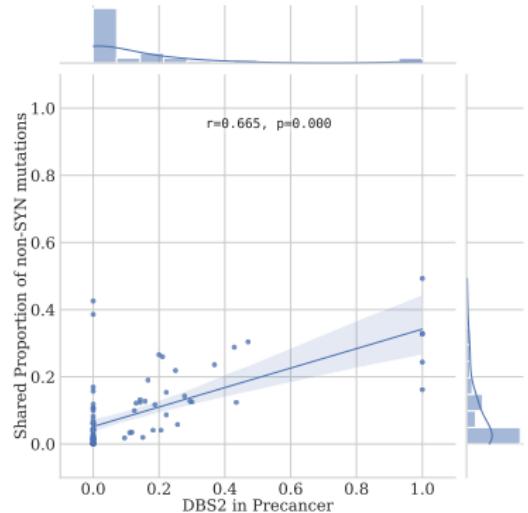
(a) Precancer score



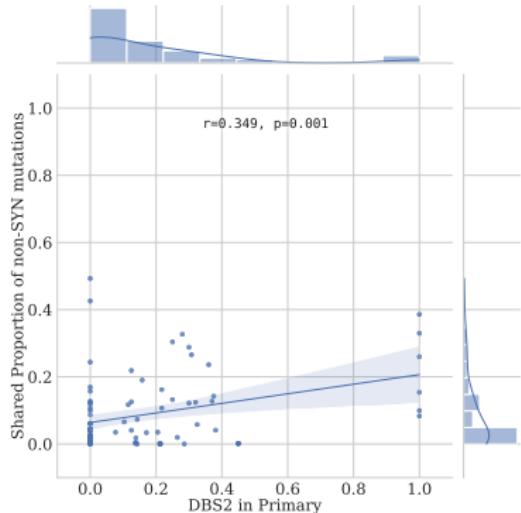
(b) Primary score

Figure: DBS2 with CIS samples

DBS2 with Shared Mutation Proportion in LUSC II



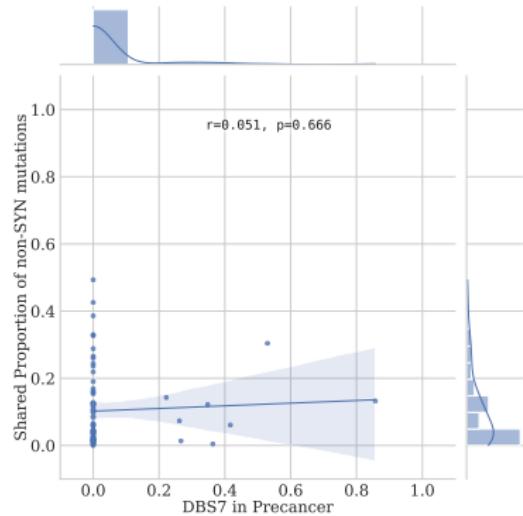
(a) Precancer score



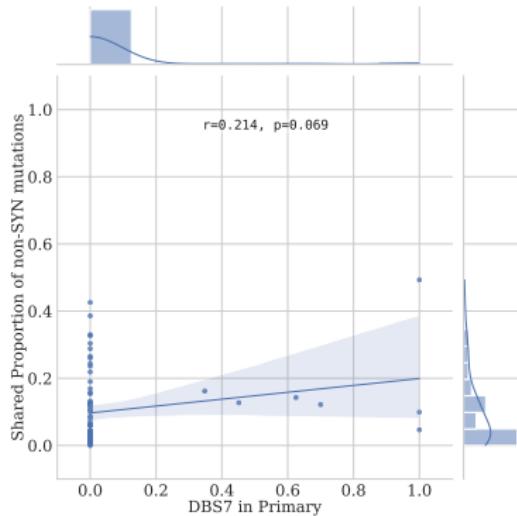
(b) Primary score

Figure: DBS2 with Precancer samples

DBS7 with Shared Mutation Proportion in LUSC I



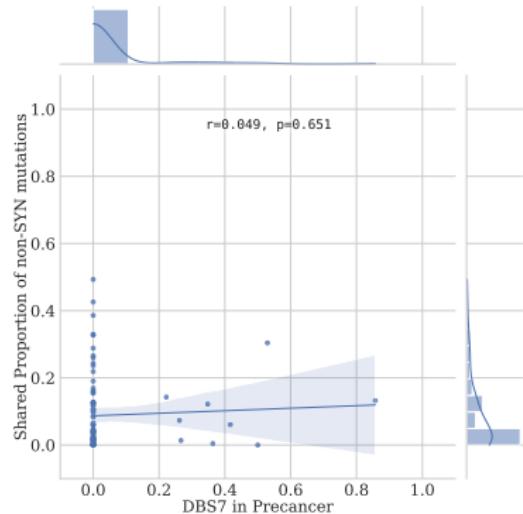
(a) Precancer score



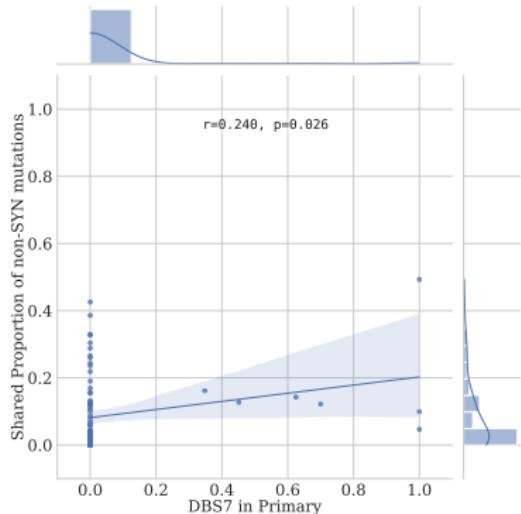
(b) Primary score

Figure: DBS7 with CIS samples

DBS7 with Shared Mutation Proportion in LUSC II



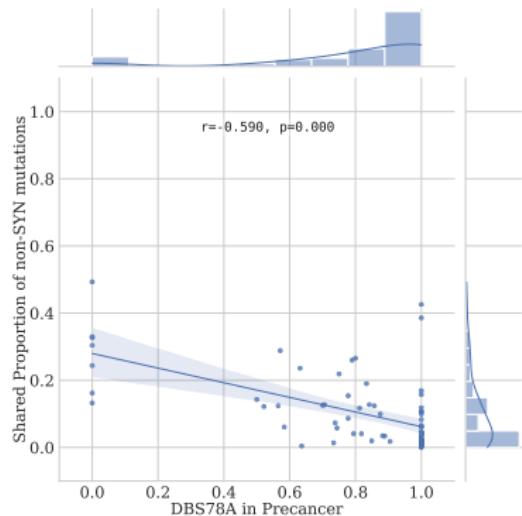
(a) Precancer score



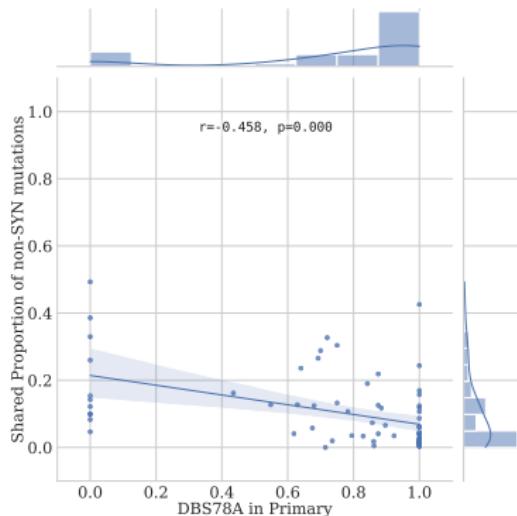
(b) Primary score

Figure: DBS7 with Precancer samples

DBS78A with Shared Mutation Proportion in LUSC I



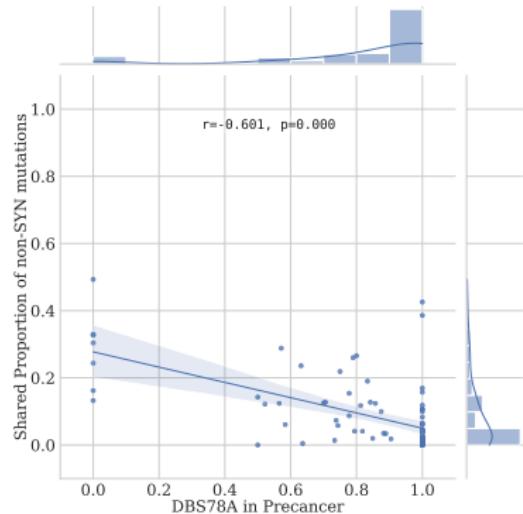
(a) Precancer score



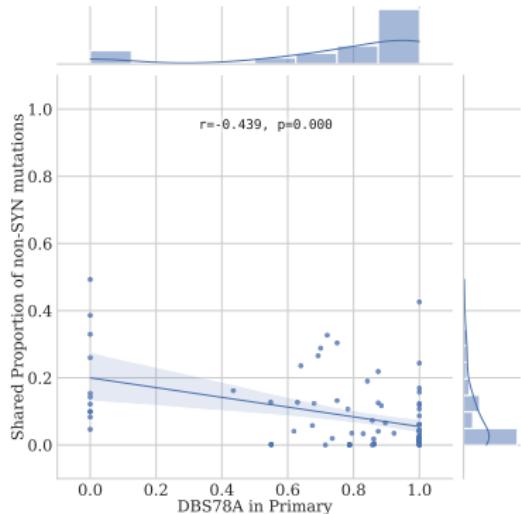
(b) Primary score

Figure: DBS78A with CIS samples

DBS78A with Shared Mutation Proportion in LUSC II



(a) Precancer score



(b) Primary score

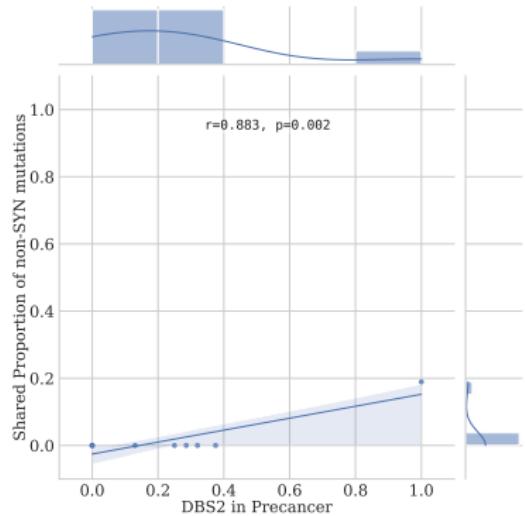
Figure: DBS78A with Precancer samples

4. Results

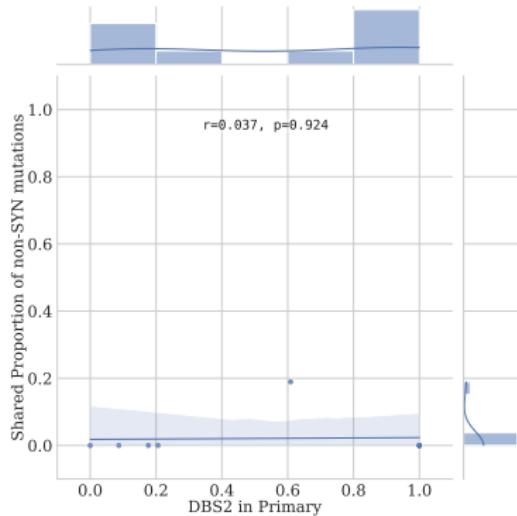
4.9. Discovery of Mutational Signature

4.9.7. DBS with Shared Mutation Proportion in LUAD

DBS2 with Shared Mutation Proportion in LUAD I



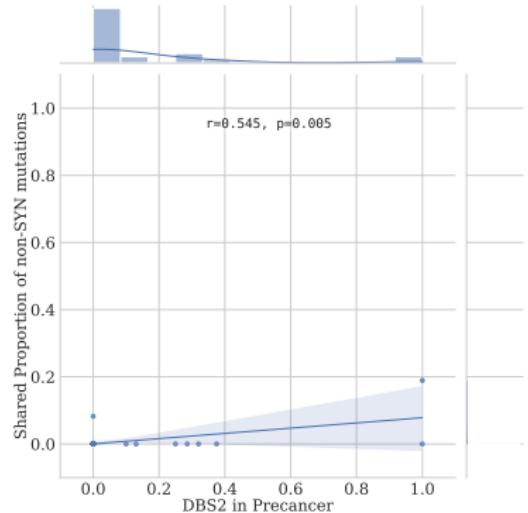
(a) Precancer score



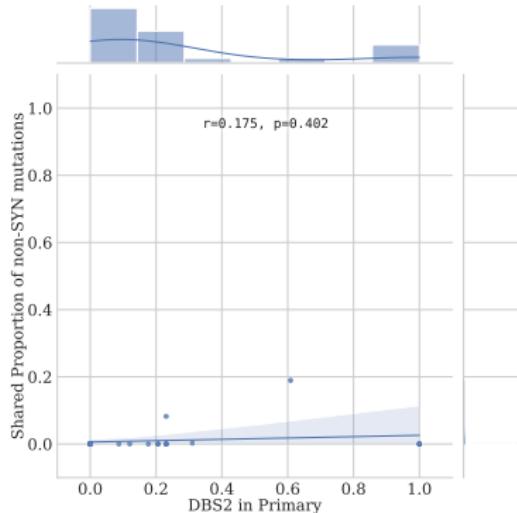
(b) Primary score

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

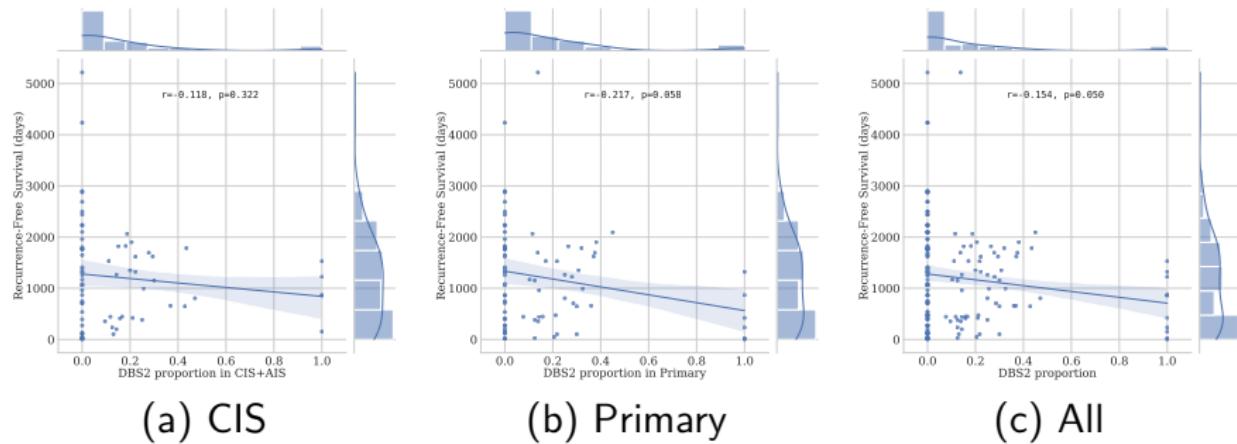


Figure: DBS2 with RFS in LUSC

DBS78A with Recurrence-Free Survival in LUSC

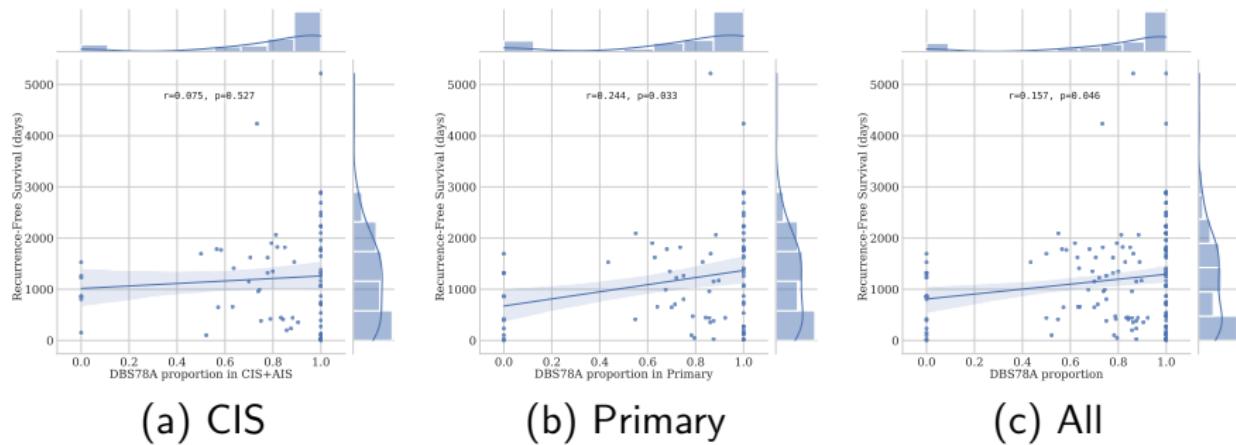
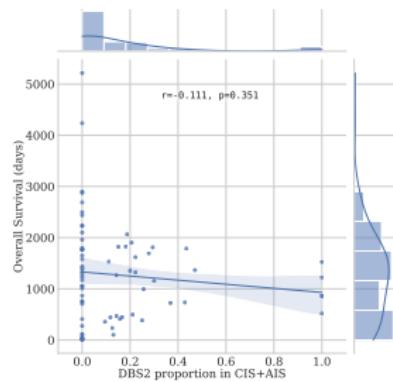
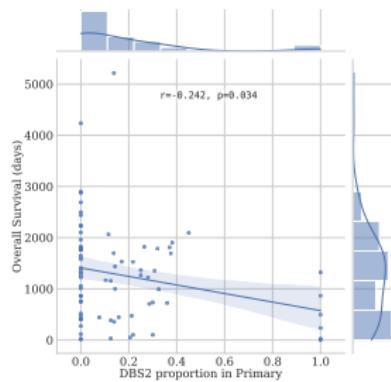


Figure: DBS78A with RFS in LUSC

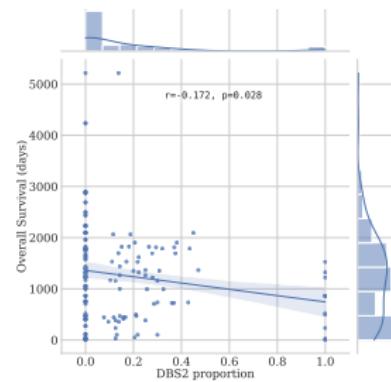
DBS2 with Overall Survival in LUSC I



(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

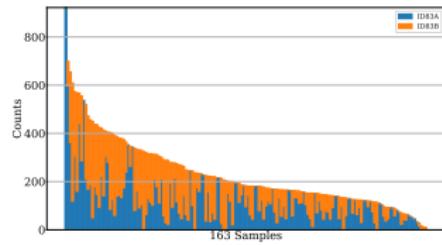
ID83A

- Unknown

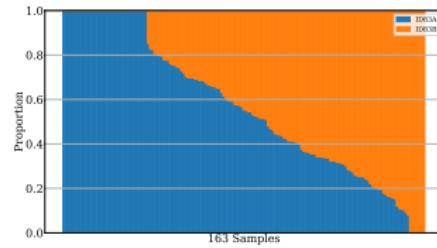
ID83B

- Unknown

Indels in LUSC I



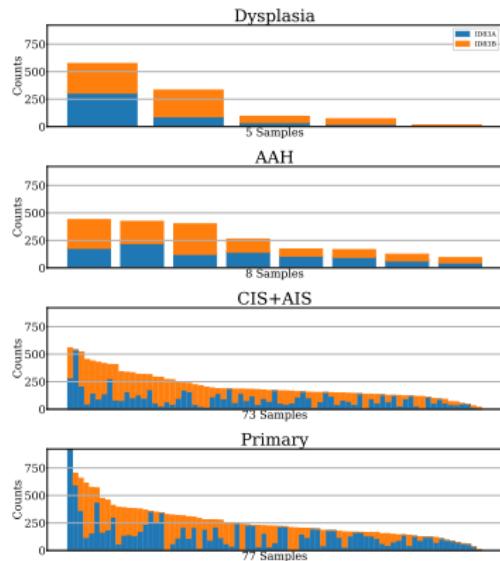
(a) Absolute



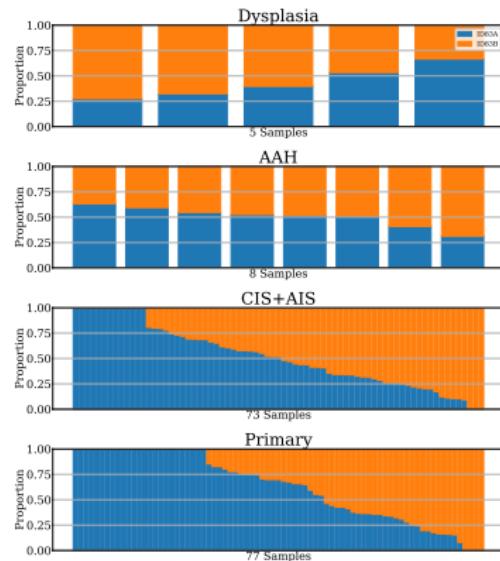
(b) Relative

Figure: Indel Bar Plot in LUSC

Indels in LUSC II



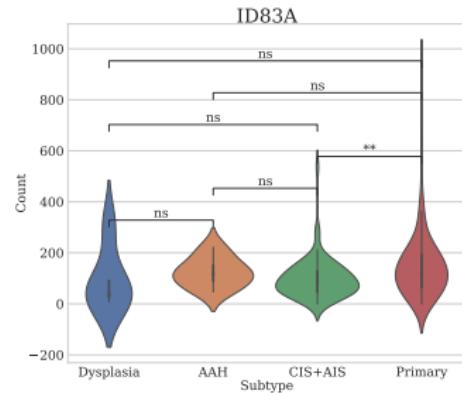
(a) Absolute



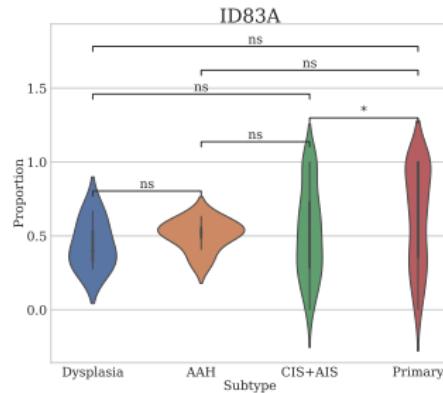
(b) Relative

Figure: Indel Bar Plot by Cancer Subtype in LUSC

Indels in LUSC III



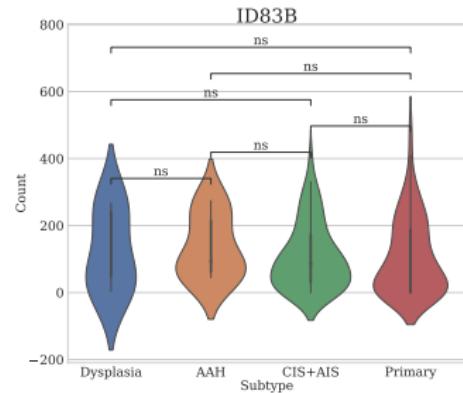
(a) Absolute



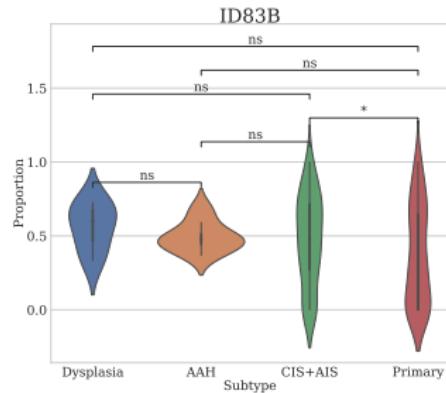
(b) Relative

Figure: Indel83A Signature in LUSC

Indels in LUSC IV



(a) Absolute



(b) Relative

Figure: Indel83B Signature in LUSC

Indel in LUSC with Smoking I

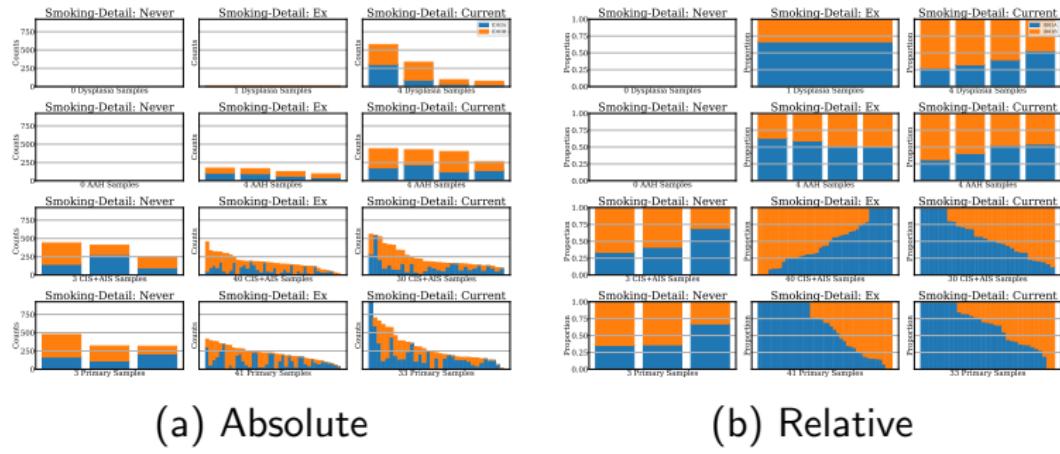
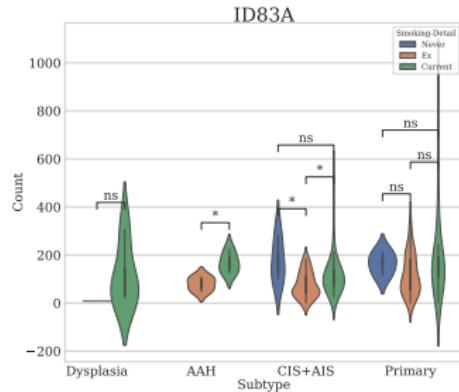
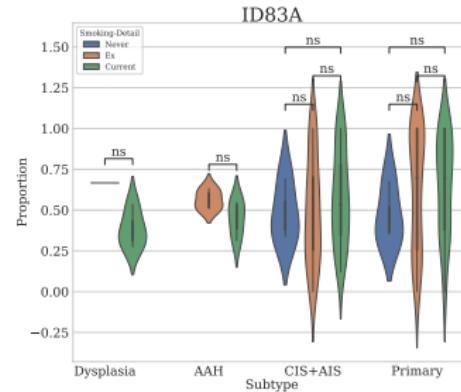


Figure: Indel Bar Plot by Cancer Subtype & Smoking in LUSC

Indel in LUSC with Smoking II



(a) Absolute



(b) Relative

Figure: Indel83A Signature in LUSC with Smoking

Indel in LUSC with Recurrence

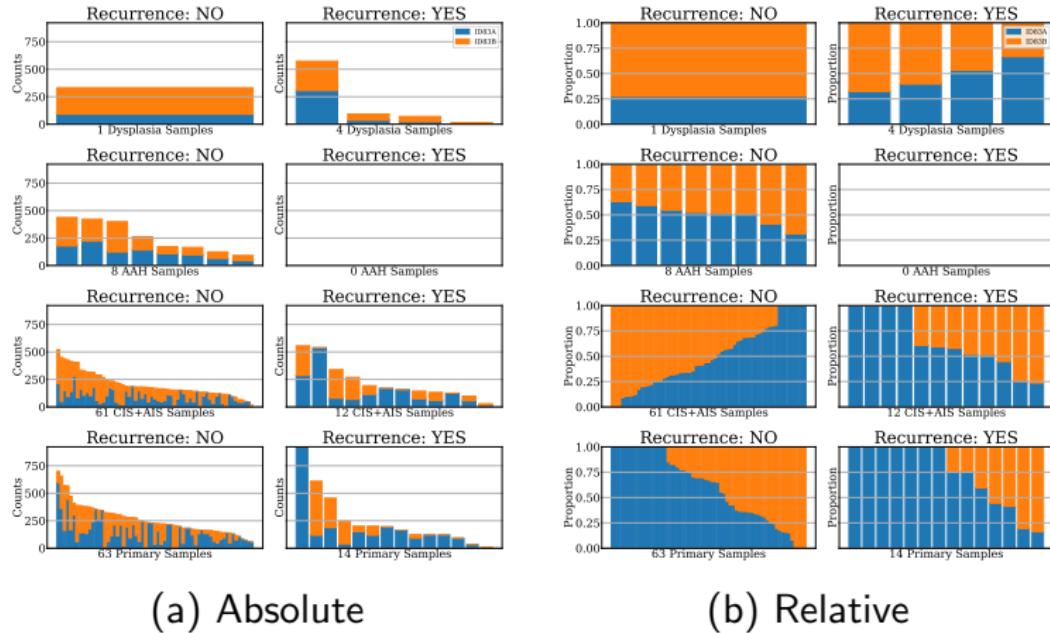
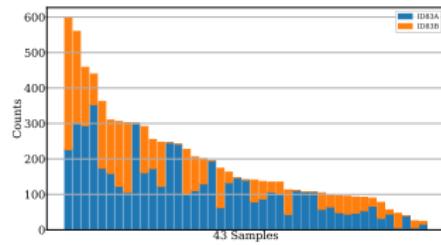
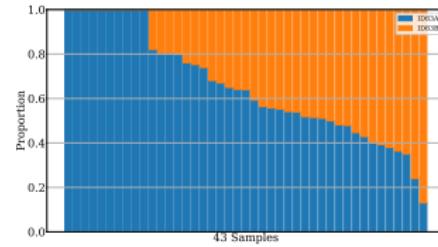


Figure: Indel Bar Plot by Cancer Subtype & Recurrence in LUSC

Indels in LUAD I



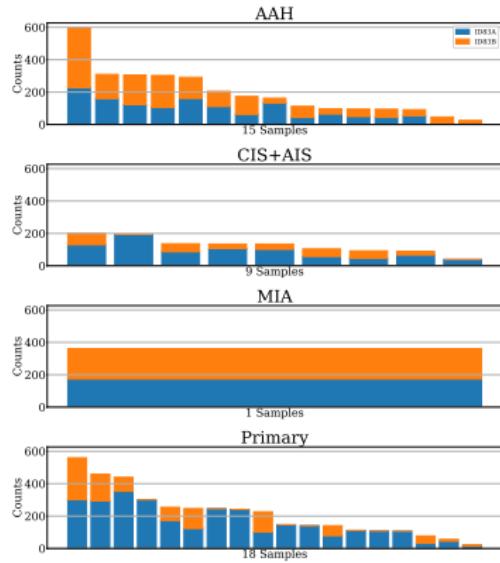
(a) Absolute



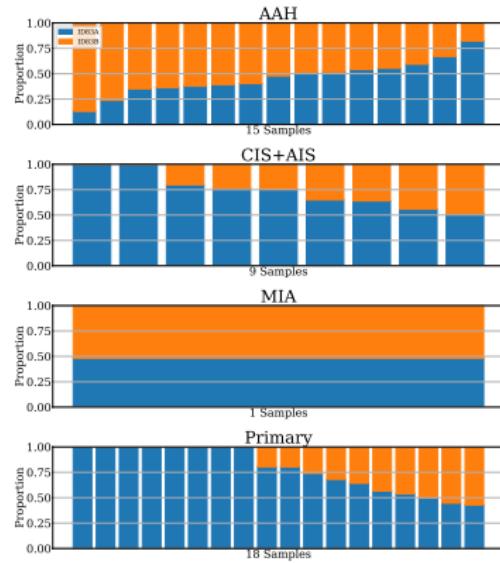
(b) Relative

Figure: Indel Bar Plot in LUAD

Indels in LUAD II



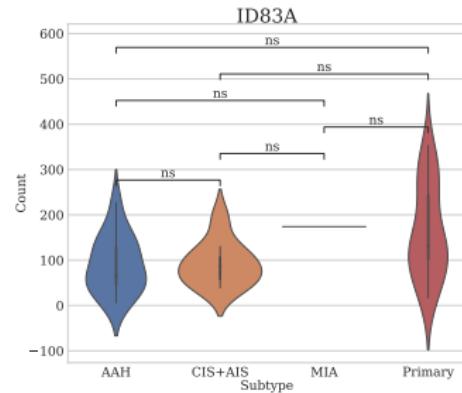
(a) Absolute



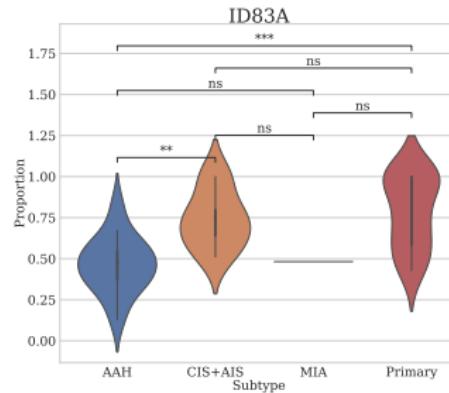
(b) Relative

Figure: Indel Bar Plot by Cancer Subtype in LUAD

Indels in LUAD III



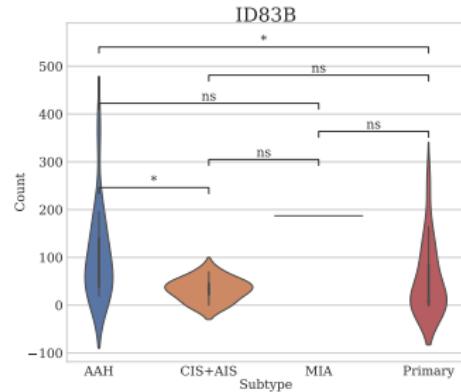
(a) Absolute



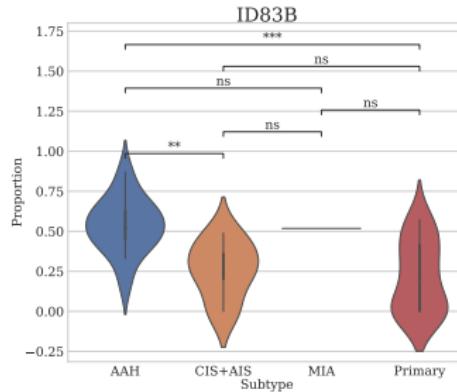
(b) Relative

Figure: Indel83A Signature in LUAD

Indels in LUAD IV



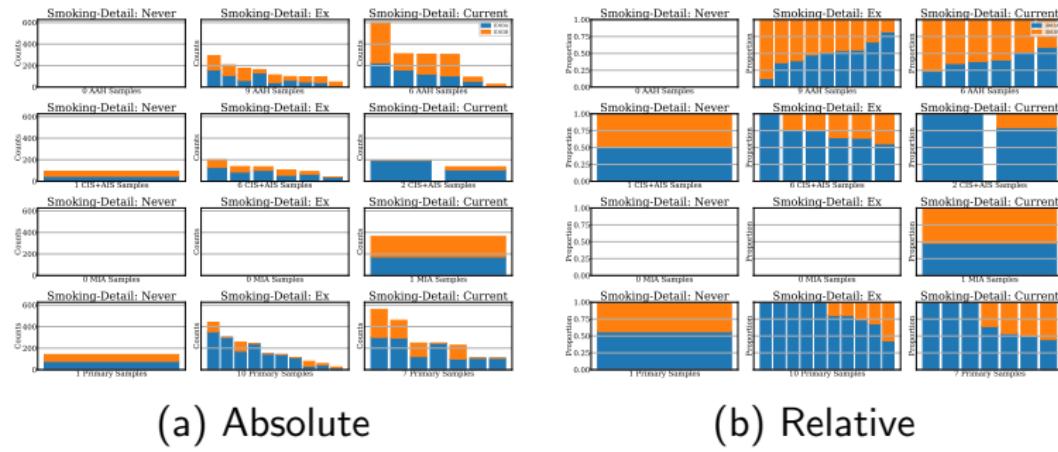
(a) Absolute



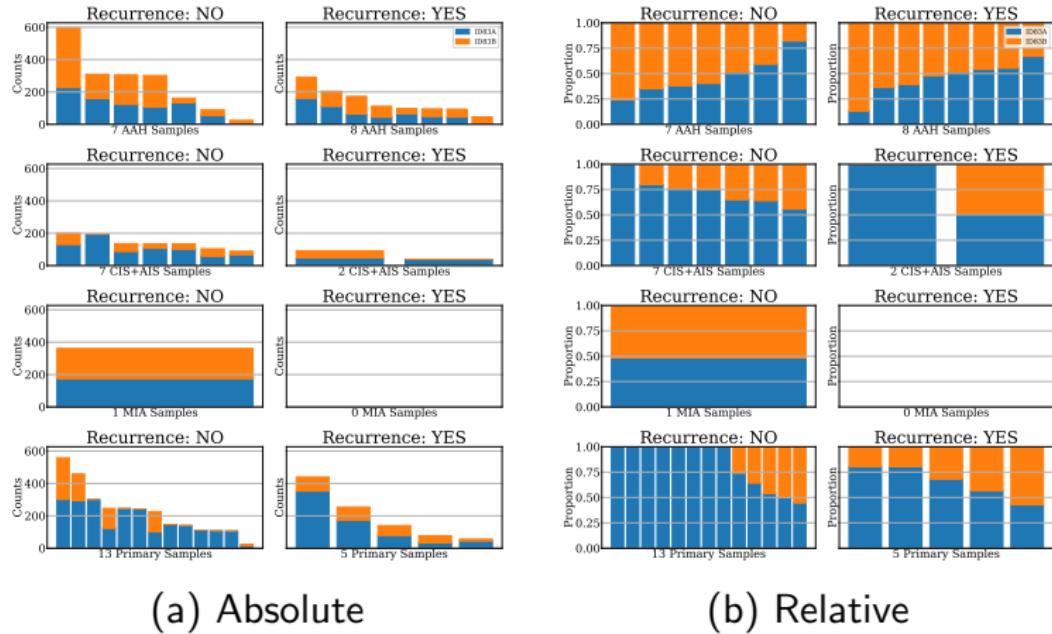
(b) Relative

Figure: Indel83B Signature in LUAD

Indel in LUAD with Smoking



Indel in LUAD with Recurrence



(a) Absolute

(b) Relative

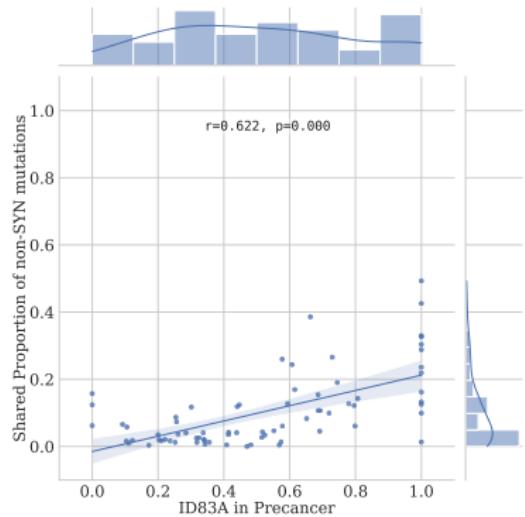
Figure: Indel Bar Plot by Cancer Subtype & Recurrence in LUAD

4. Results

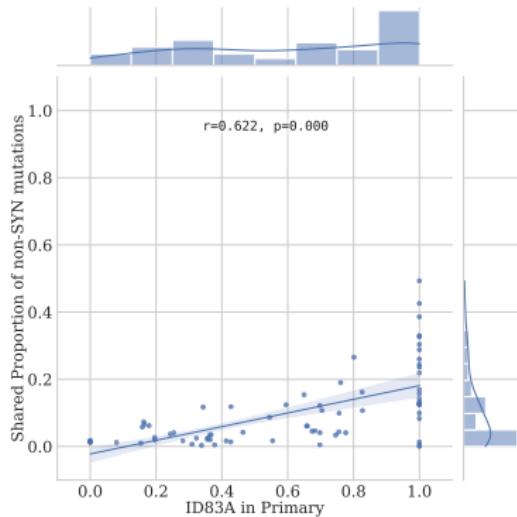
4.9. Discovery of Mutational Signature

4.9.10. Indel with Shared mutation Proportion in LUSC

ID83A with Shared Mutation Proportion in LUSC I



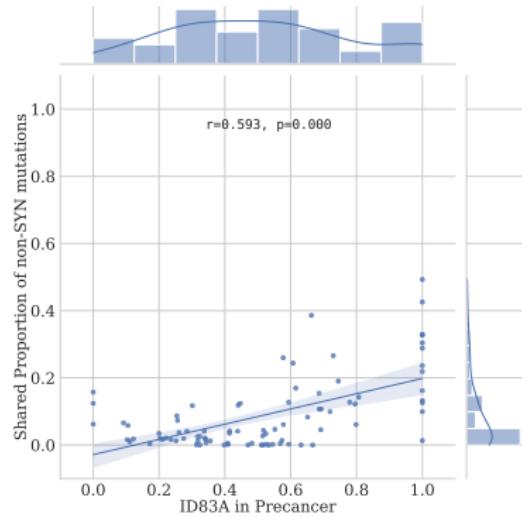
(a) Precancer score



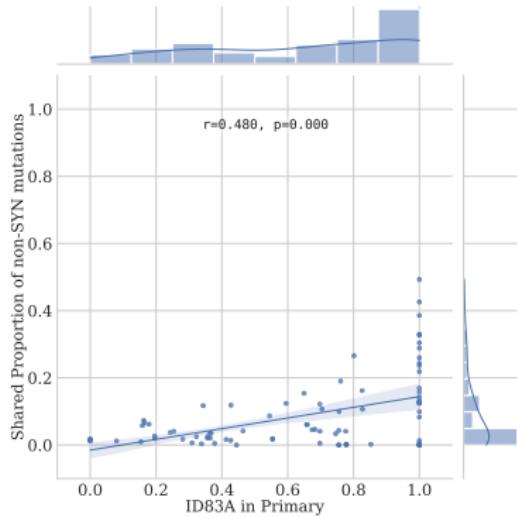
(b) Primary score

Figure: ID83A with CIS samples

ID83A with Shared Mutation Proportion in LUSC II



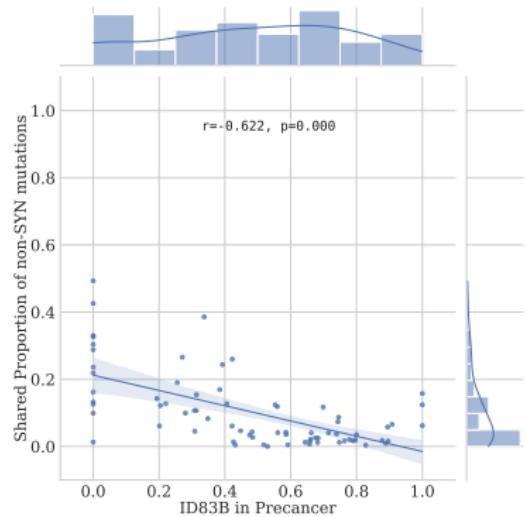
(a) Precancer score



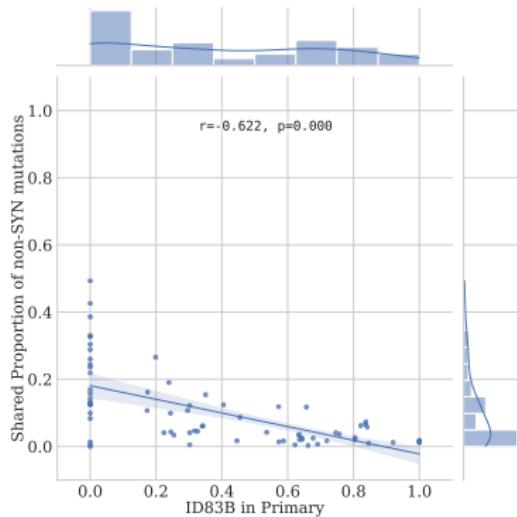
(b) Primary score

Figure: ID83A with Precancer samples

ID83B with Shared Mutation Proportion in LUSC I



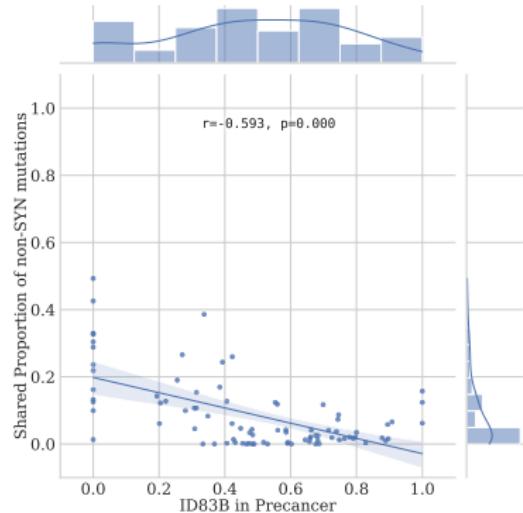
(a) Precancer score



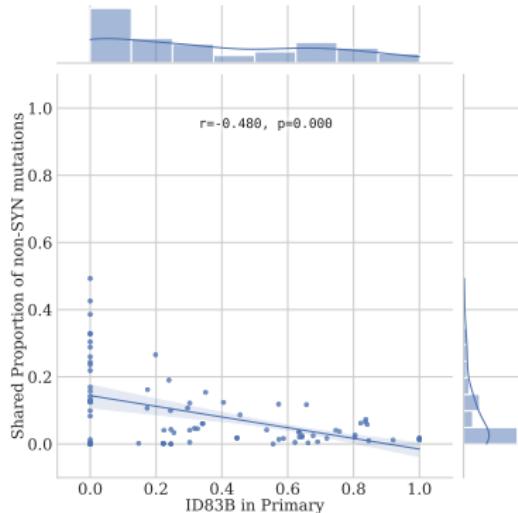
(b) Primary score

Figure: ID83B with CIS samples

ID83B with Shared Mutation Proportion in LUSC II



(a) Precancer score



(b) Primary score

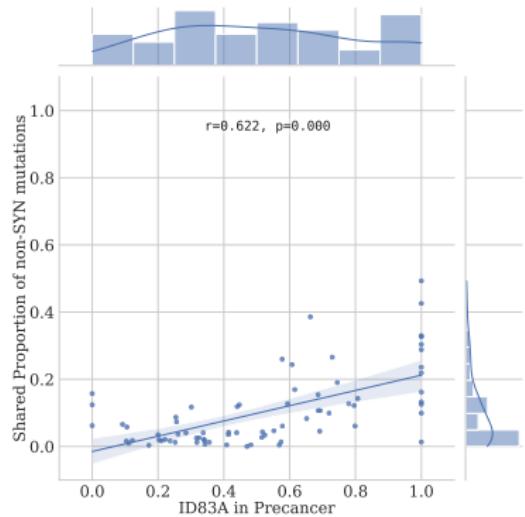
Figure: ID83B with Precancer samples

4. Results

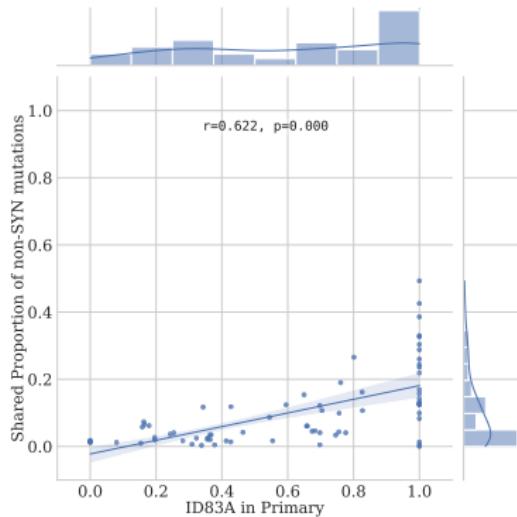
4.9. Discovery of Mutational Signature

4.9.11. Indel with Shared Mutation Proportion in LUSC

ID83A with Shared Mutation Proportion in LUSC I



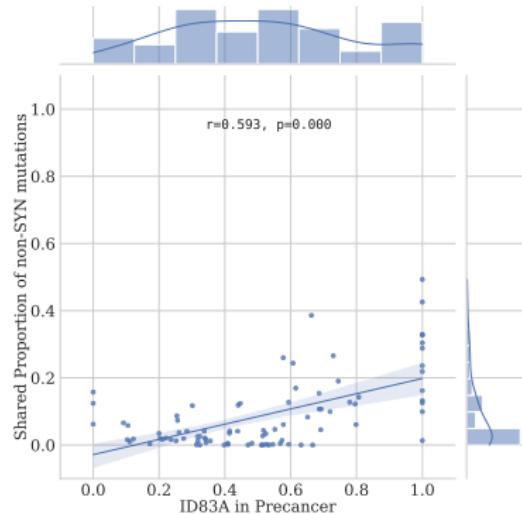
(a) Precancer score



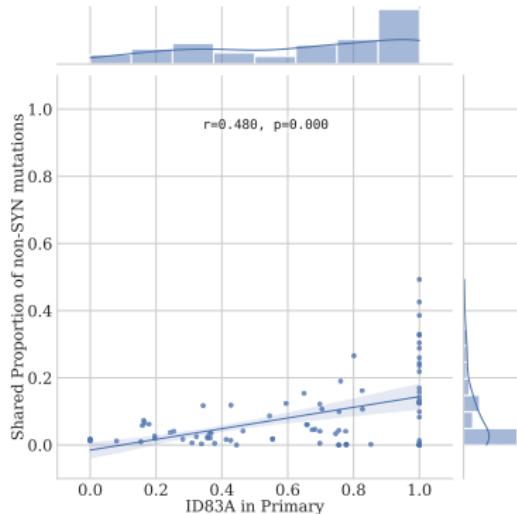
(b) Primary

Figure: ID83A with CIS samples

ID83A with Shared Mutation Proportion in LUSC II



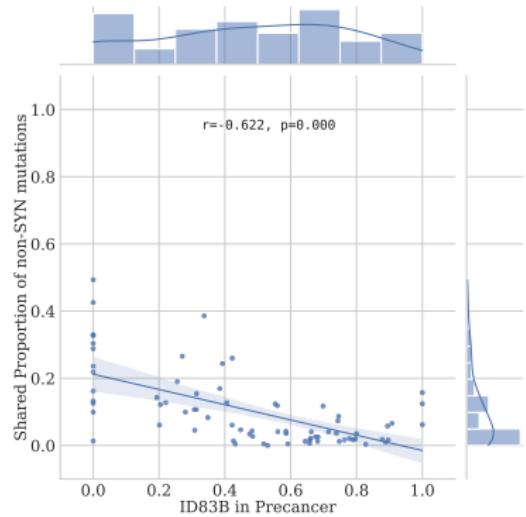
(a) Precancer score



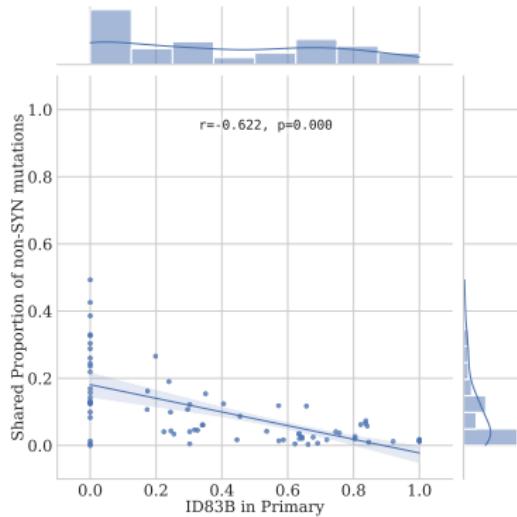
(b) Primary

Figure: ID83A with Precancer samples

ID83B with Shared Mutation Proportion in LUSC I



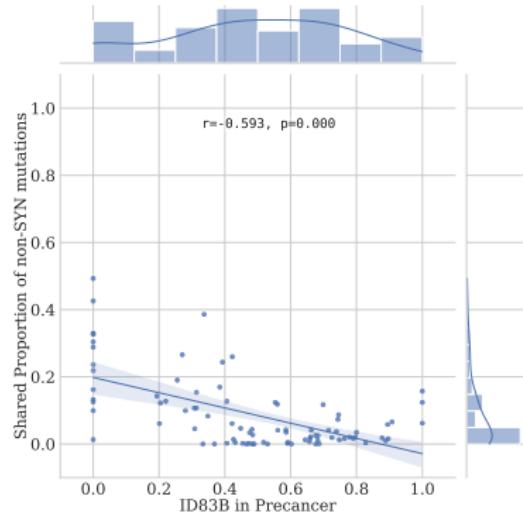
(a) Precancer score



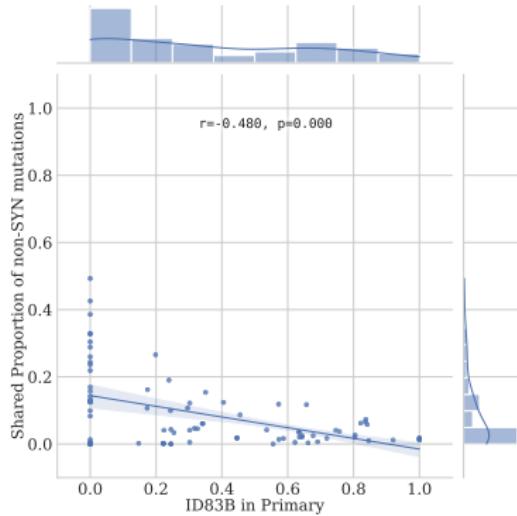
(b) Primary

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?

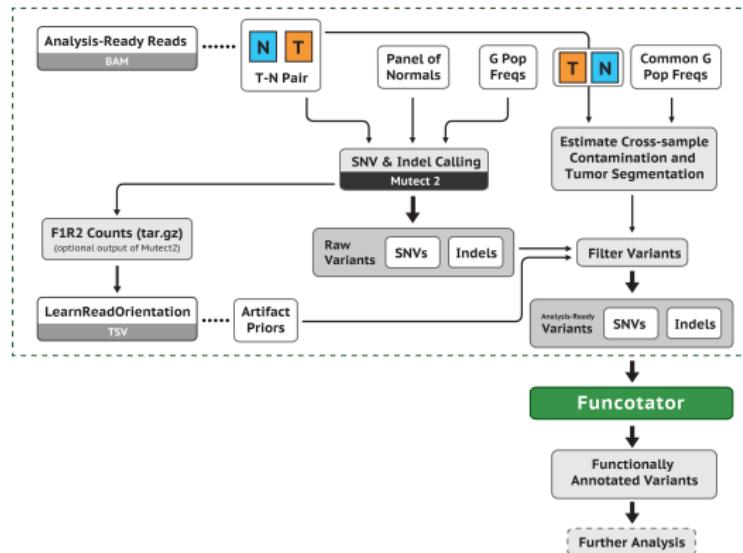


Figure: Somatic short variant discovery workflow (Van der Auwera et al., 2013; DePristo et al., 2011)

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

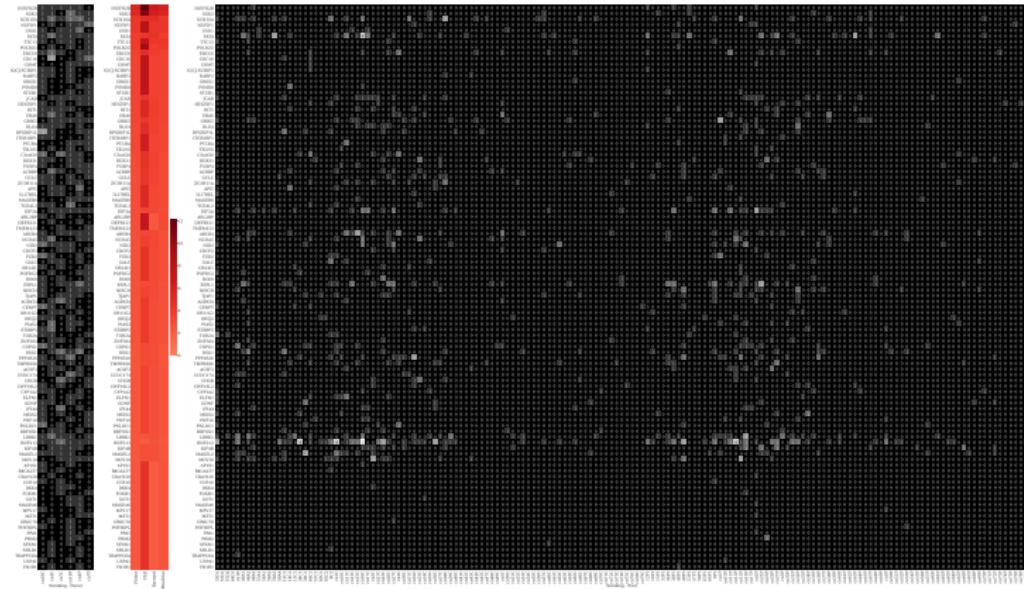


Figure: Clinical Data about LUSC for Smoking

Clinical Data about LUSC for Smoking II

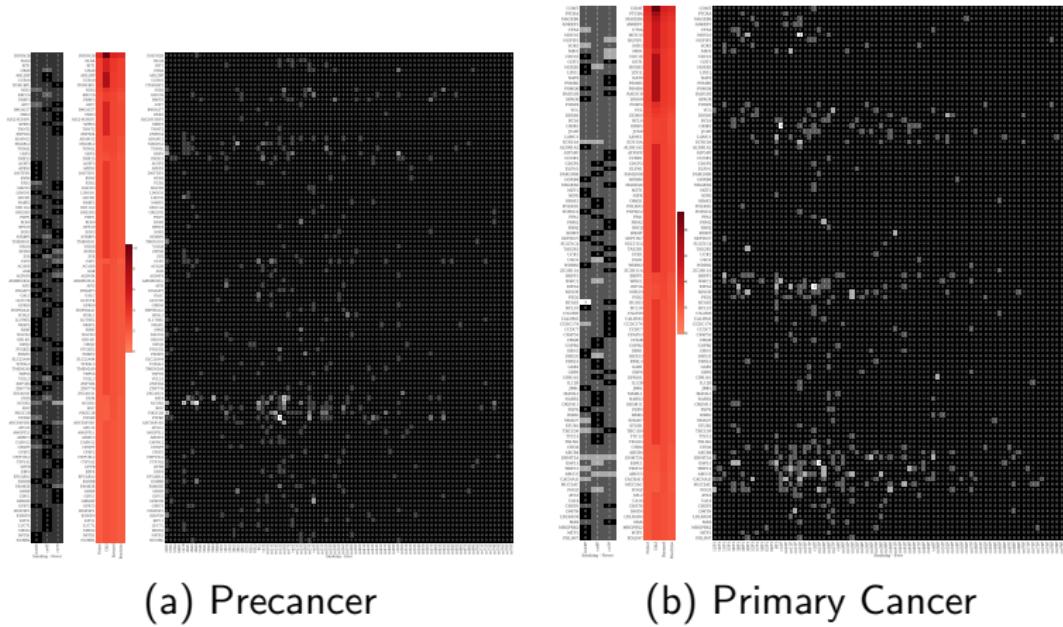


Figure: Clinical Data about LUSC for Smoking with Precancer/Primary

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

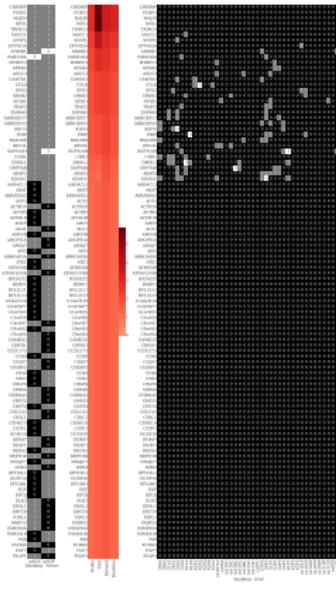
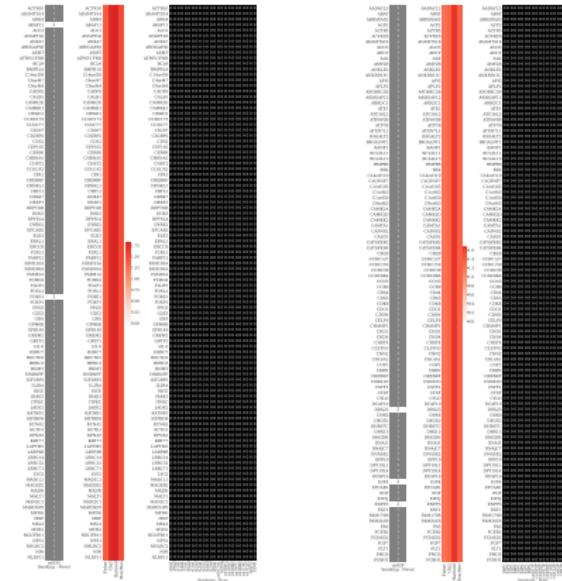


Figure: Clinical Data about LUAD for Smoking

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

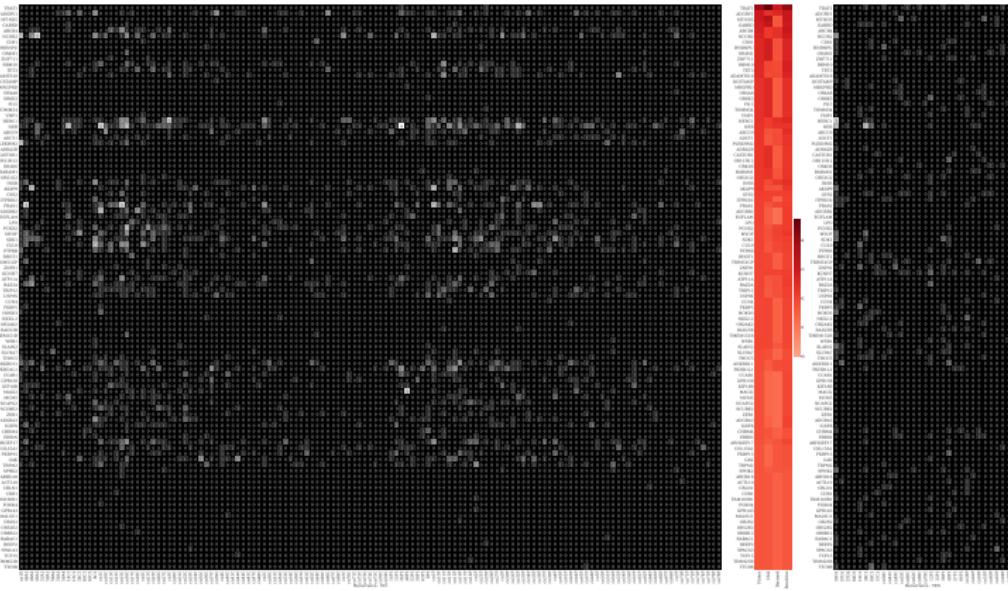


Figure: Clinical Data about LUSC for Recurrence

Clinical Data about LUSC for Recurrence II

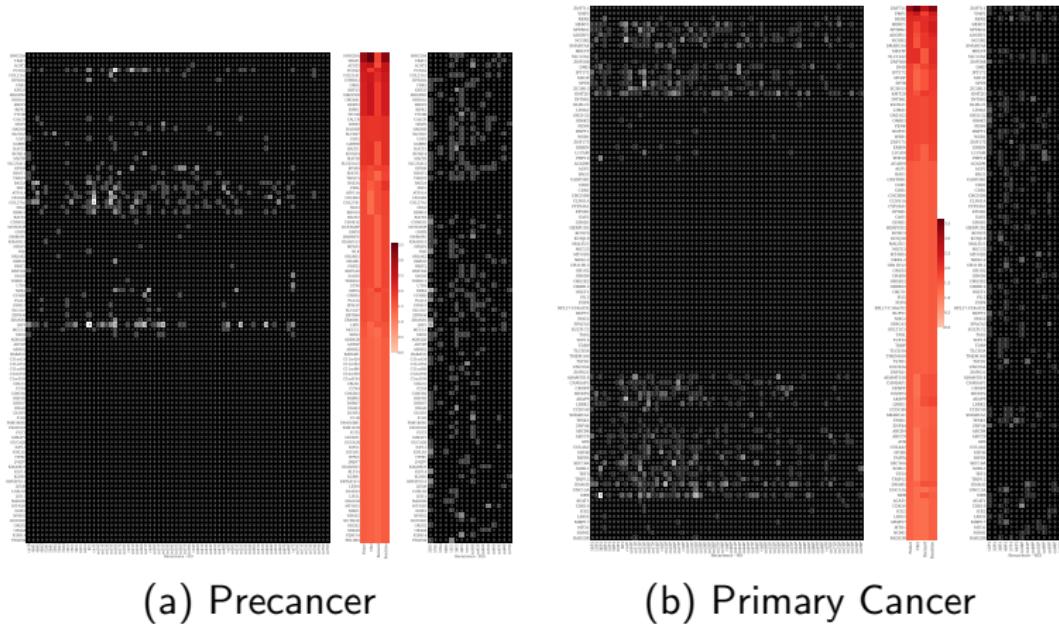


Figure: Clinical Data about LUSC for Recurrence with Precancer/Primary

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

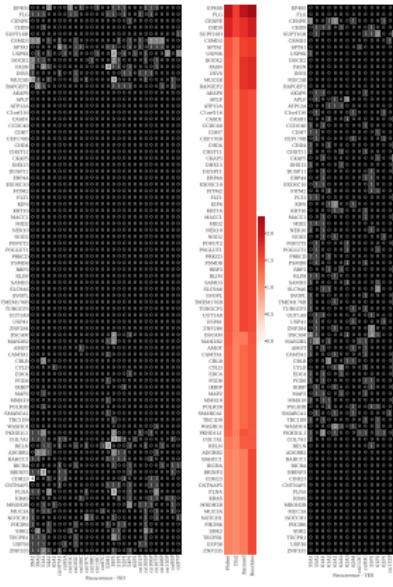
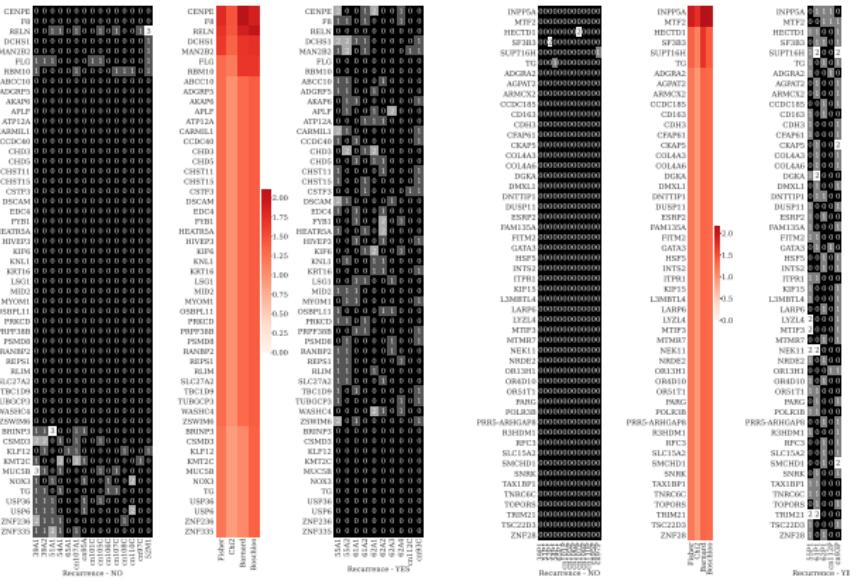


Figure: Clinical Data about LUAD for Recurrence

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?

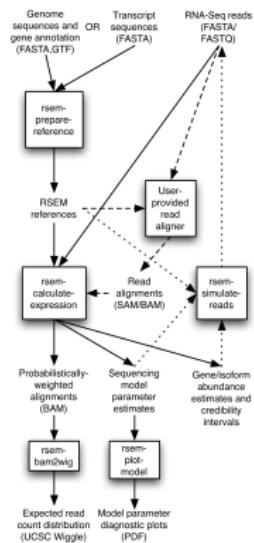


Figure: RSEM workflow (B. Li & Dewey, 2011)

DESeq2?

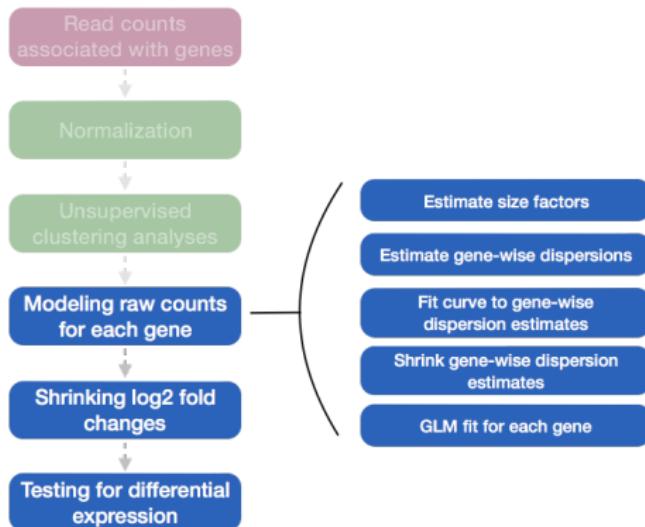


Figure: DESeq2 workflow (Love, Huber, & Anders, 2014)

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?

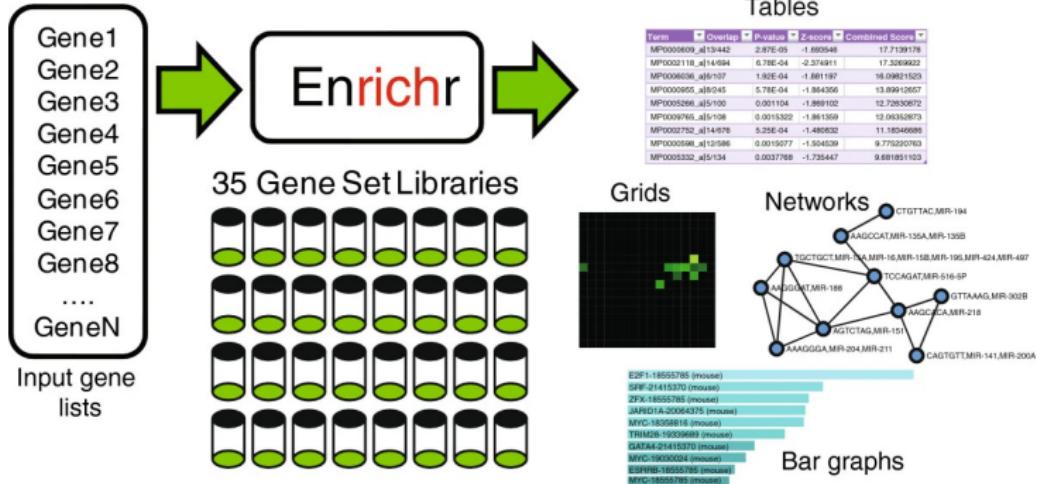


Figure: Enrichr workflow (E. Y. Chen et al., 2013; Kuleshov et al., 2016)

Gene-set Library

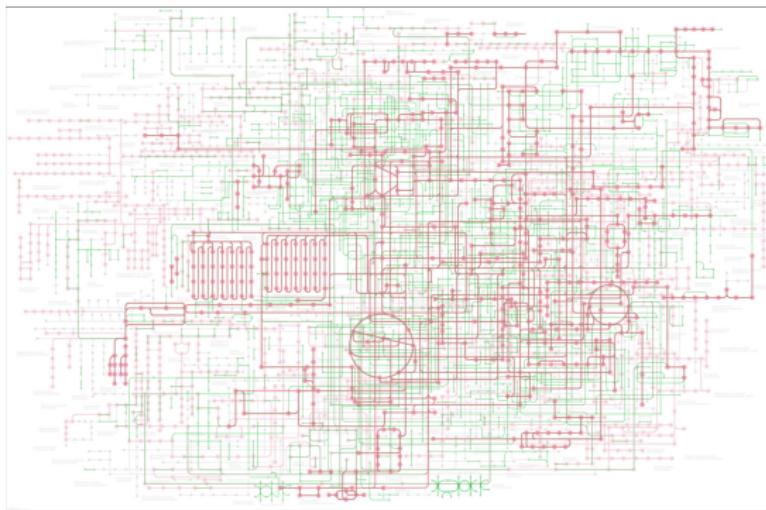


Figure: The global map of metabolic pathways by KEGG (Kanehisa et al., 2021)

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	

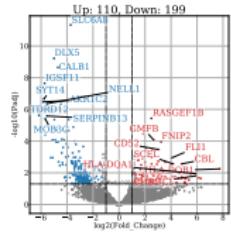
4. Results

4.11. Differences in Gene Expression Levels

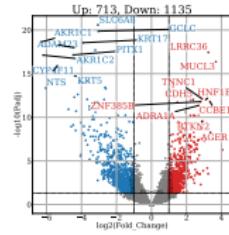
4.11.1. Comparing cancer stage in LUSC

DEG Volcano Plots in LUSC

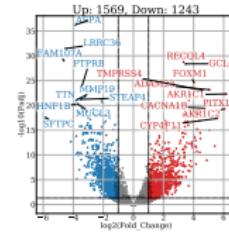
Normal → Dysplasia → CIS → Primary (LUSC)



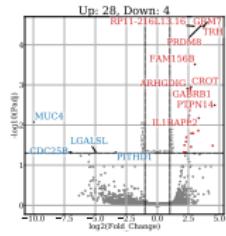
(a) Normal-Dysplasia



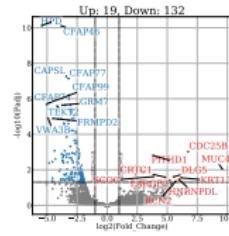
(b) Normal-CIS



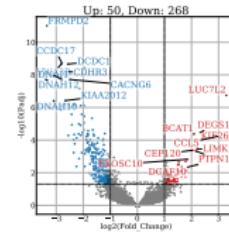
(c) Normal-Primary



(d) Dysplasia-CIS



(e) Dysplasia-Primary

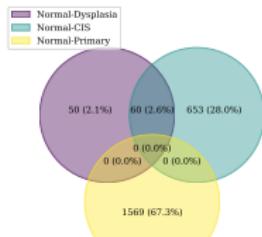


(f) CIS-Primary

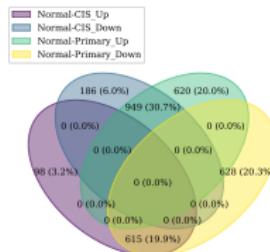
Figure: DEG Volcano Plots in LUSC

DEG Venn Diagram in LUSC

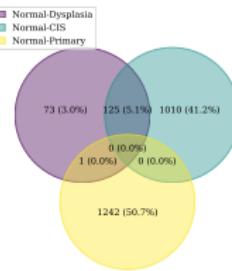
Normal → Dysplasia → CIS → Primary (LUSC)



(a) Up-regulated



(b) Both



(c) Down-regulated

Figure: DEG Venn Diagram in LUSC

Enrichment test with Normal vs. Dysplasia in LUSC

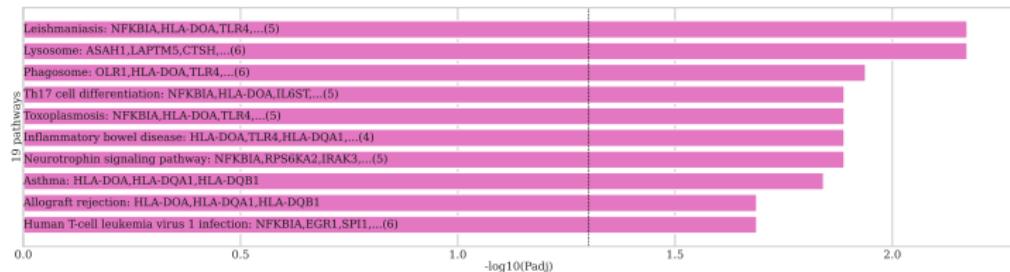


Figure: Up-regulated Pathways on Normal vs. Dysplasia

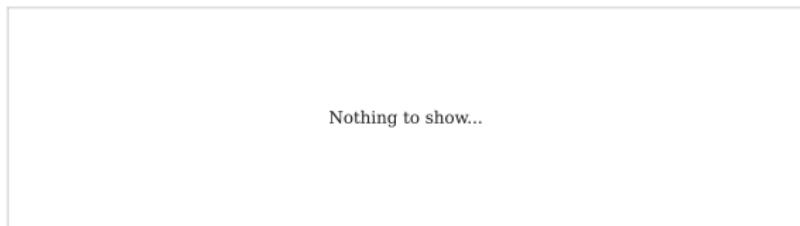


Figure: Down-regulated Pathways on Normal vs. Dysplasia

Enrichment test with Normal vs. CIS in LUSC

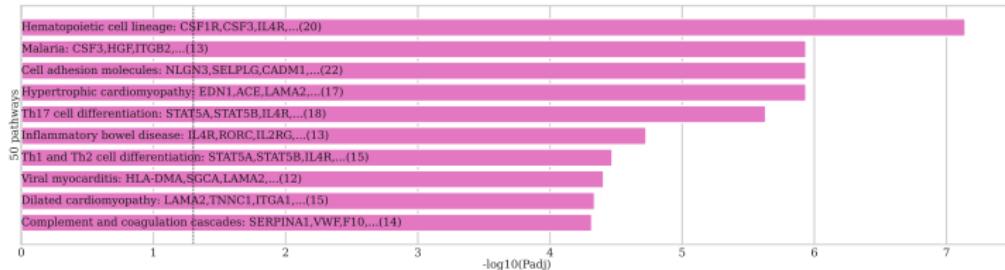


Figure: Up-regulated Pathways on Normal vs. CIS

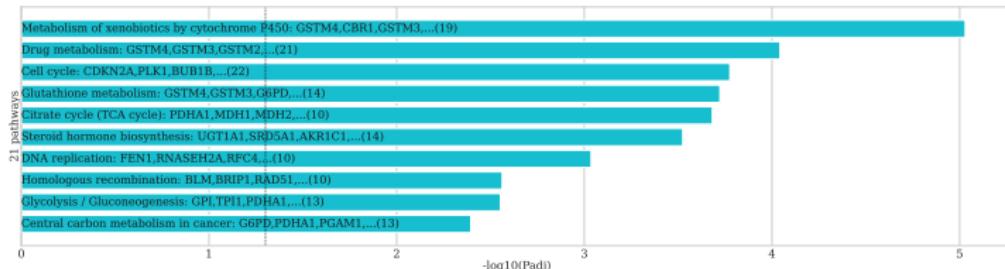


Figure: Down-regulated Pathways on Normal vs. CIS

Enrichment test with Normal vs. Primary in LUSC

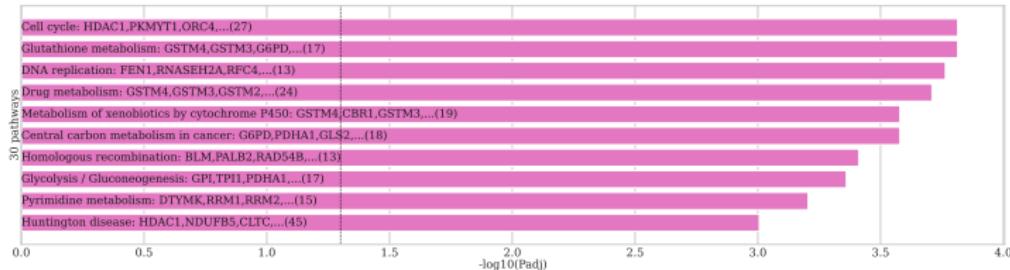


Figure: Up-regulated Pathways on Normal vs. Primary

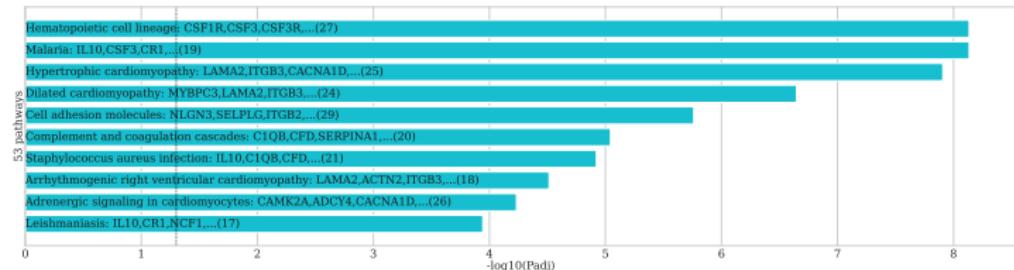


Figure: Down-regulated Pathways on Normal vs. Primary

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).

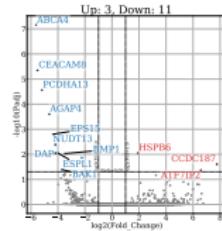
4. Results

4.11. Differences in Gene Expression Levels

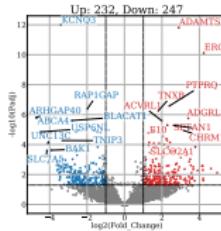
4.11.2. Comparing cancer stage in LUAD

DEG Volcano Plots in LUAD

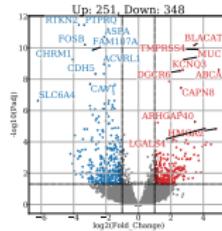
Normal → AAH → AIS → Primary (LUAD)



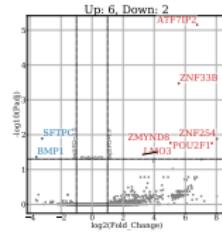
(a) Normal-AAH



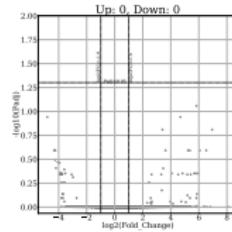
(b) Normal-AIS



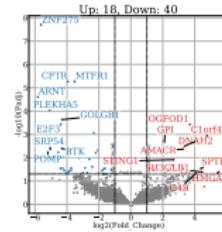
(c) Normal-Primary



(d) AAH-AIS



(e) AAH-Primary

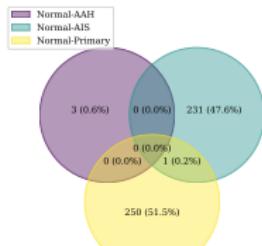


(f) AIS-Primary

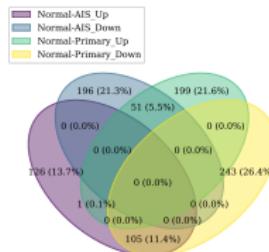
Figure: DEG Volcano Plots in LUAD

DEG Venn Diagram in LUAD

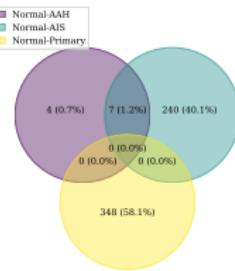
Normal → AAH → AIS → Primary (LUAD)



(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

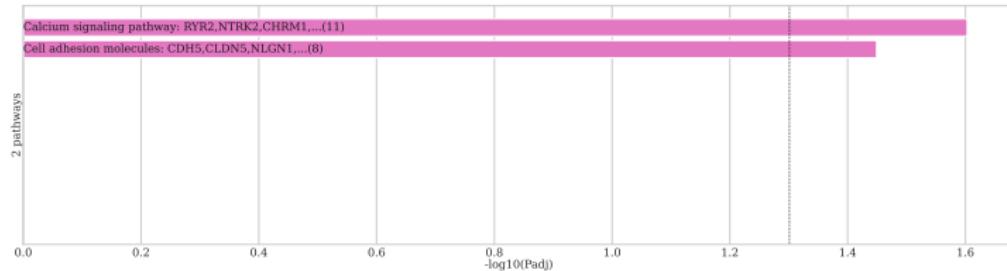


Figure: Up-regulated Pathways on Normal vs. AIS

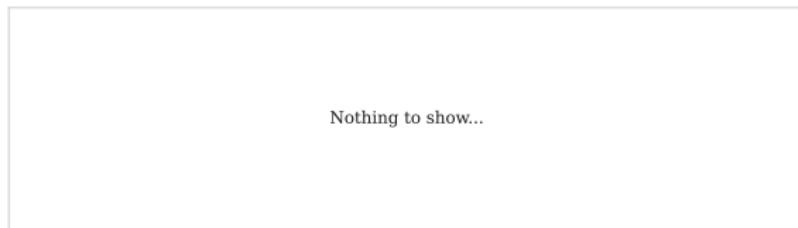


Figure: Down-regulated Pathways on Normal vs. AIS

Enrichment test with Normal vs. Primary in LUAD



Figure: Up-regulated Pathways on Normal vs. Primary

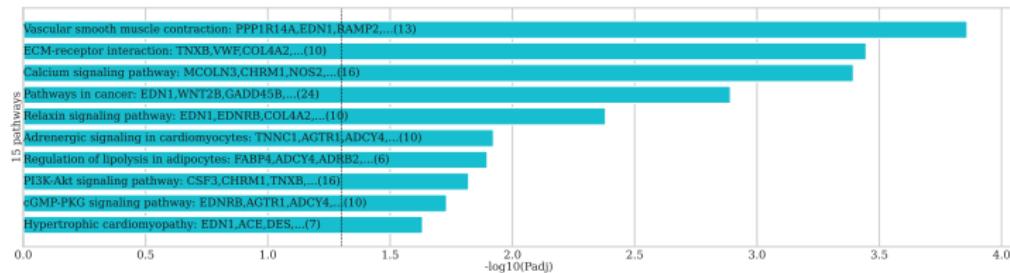


Figure: Down-regulated Pathways on Normal vs. Primary

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

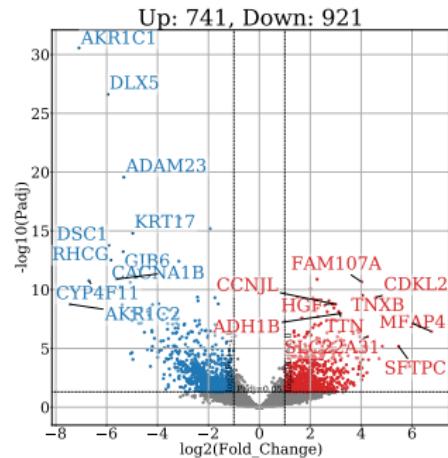
Recurrence?	Stage	Number of Samples	
		Normal	Dysplasia
Recurrence	Normal	1	
	Dysplasia		1
	CIS+AIS	5	
	Primary	6	
	Total	13	
Non-recurrence	Normal	16	
	Dysplasia		1
	CIS+AIS	29	
	Primary	30	
	Total	76	

Pooled normal samples

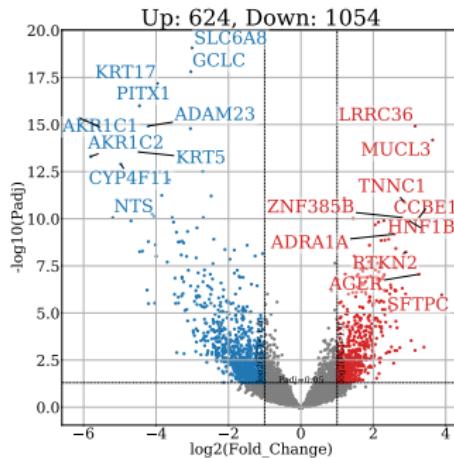
In order to compare with Normal stage, merging Normal samples.

∴ 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

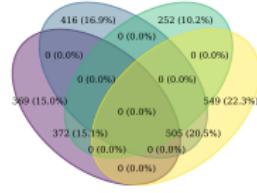
DEG Venn Diagram for R vs. NR with CIS in LUSC

Recur
Non-recur



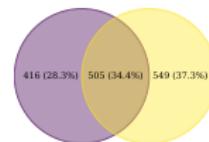
(a) Up-regulated

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

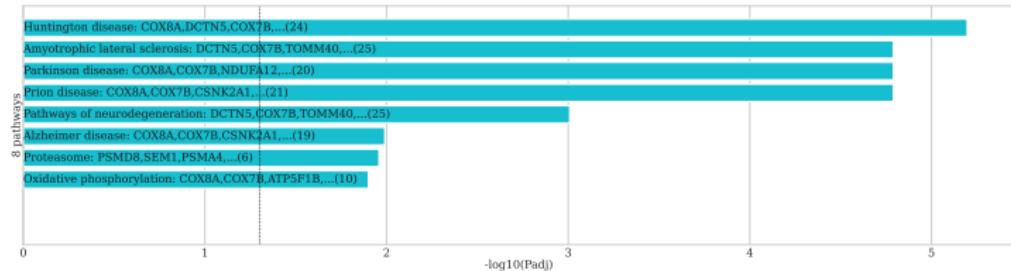


Figure: Down-regulated Pathways for Recur-specific

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

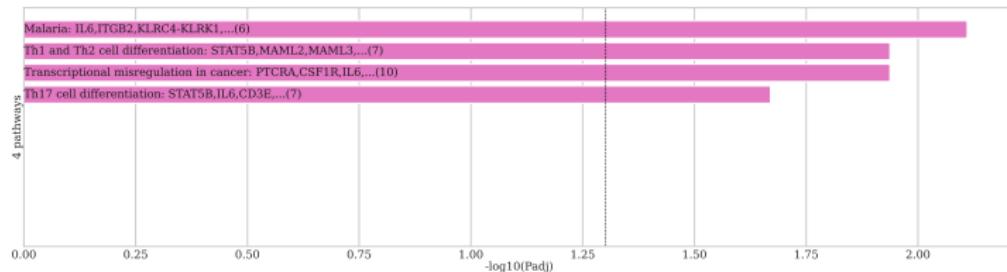


Figure: Up-regulated Pathways for Non-recr-specific



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

Enrichment test for Intersected with CIS in LUSC

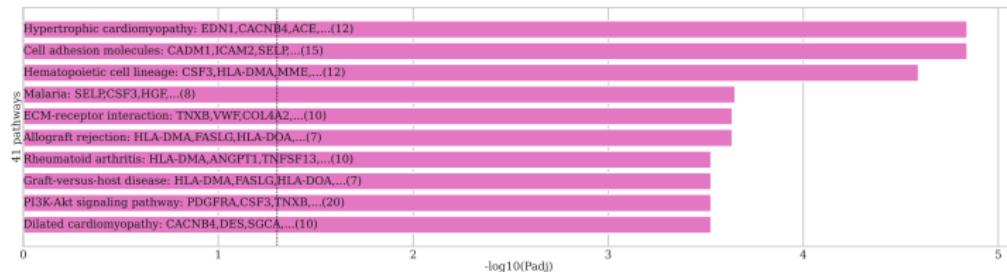


Figure: Up-regulated Pathways for Intersected

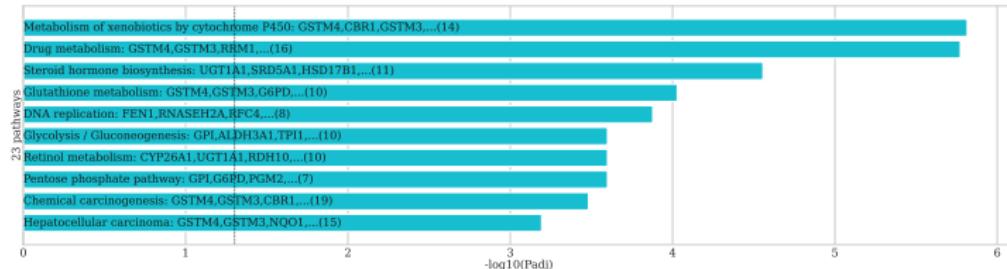
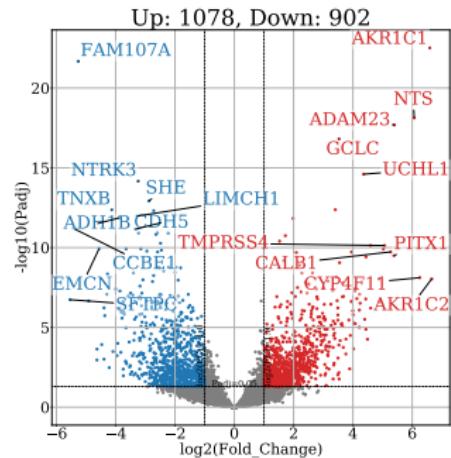
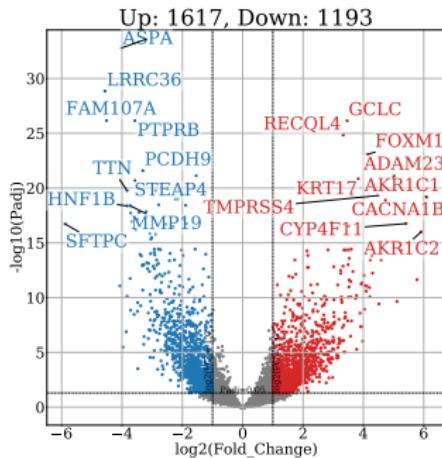


Figure: Down-regulated Pathways for Intersected

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



(a) Recur

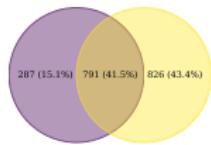


(b) Non-recur

Figure: DEG Volcanot Plot with Primary in LUSC

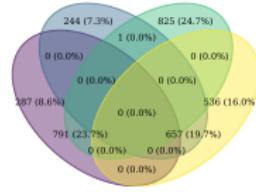
DEG Venn Diagram for R vs. NR with Primary in LUSC

Recur
Non-recur



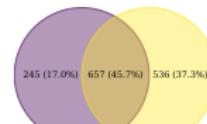
(a) Up-regulated

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

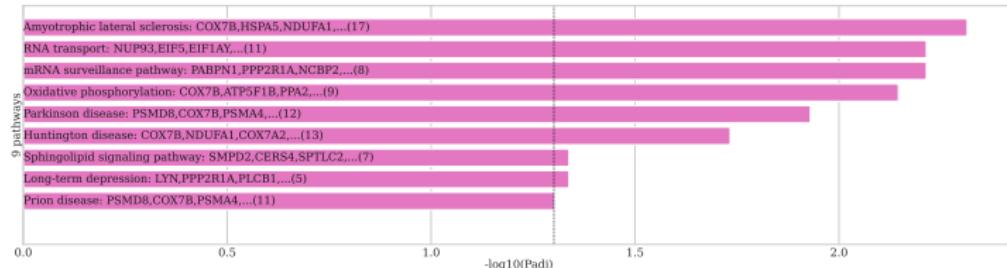


Figure: Up-regulated Pathways for Recur-specific



Figure: Down-regulated Pathways for Recur-specific

Enrichment test for NR-specific with Primary in LUSC

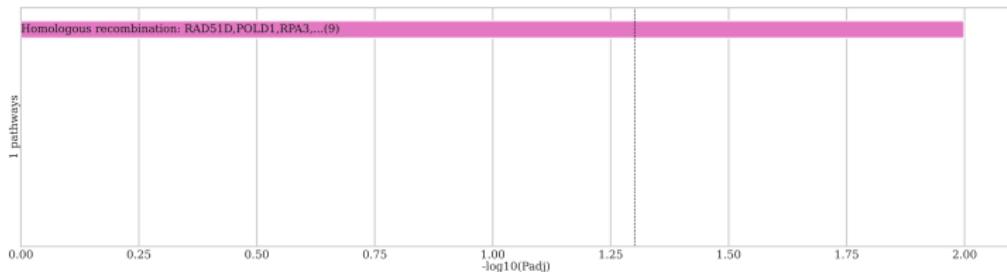


Figure: Up-regulated Pathways for Non-recur-specific

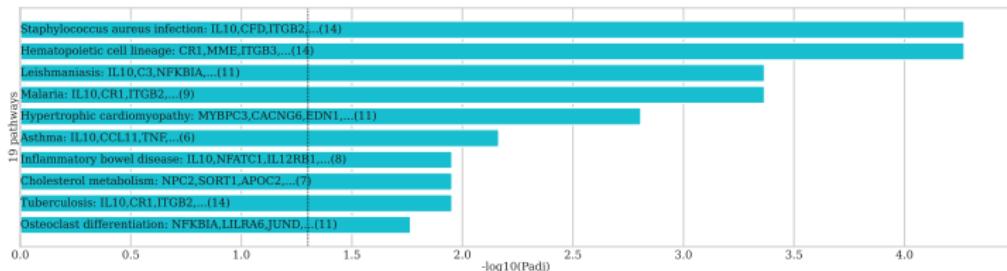


Figure: Down-regulated Pathways for Non-recur-specific

Enrichment test for Intersected with Primary in LUSC

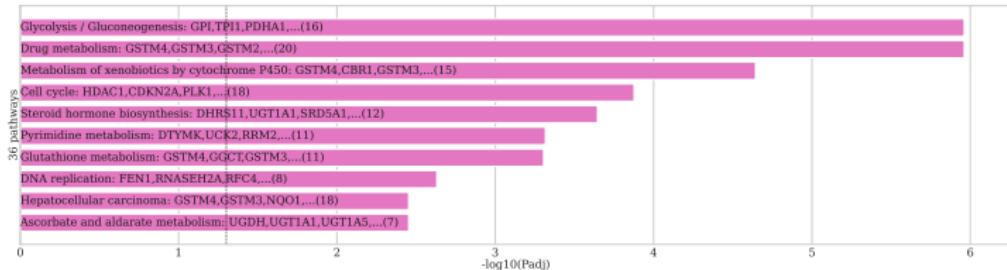


Figure: Up-regulated Pathways for Intersected

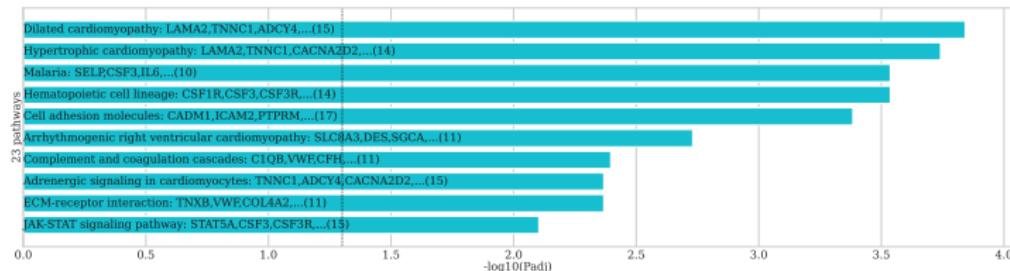


Figure: Down-regulated Pathways for Intersected

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

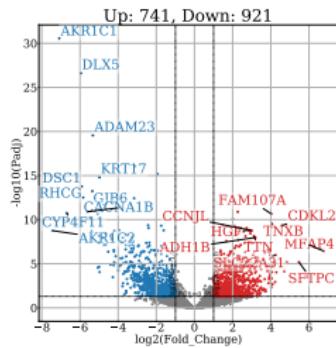
- ① Highly up-regulated in Non-recur patients.
- ② DNA-dependent ATPase (Yin, Kwon, Varshavsky, & Wang, 2004)
- ③ RECQL4 modulate chromosome segregation (Yin et al., 2004)
- ④ RECQL5 promotes metastasis & resistance in NSCLC (Xia, Zhang, Yuan, & Niu, 2021)
- ⑤ RECQL4 ↑ ⇒ Poor prognosis in breast cancer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

4. Results

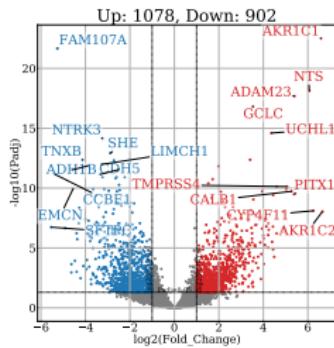
4.11. Differences in Gene Expression Levels

4.11.4. Within Recur in LUSC

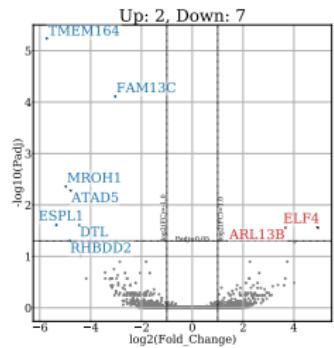
DEG Volcano Plots with Recur in LUSC



(a) Normal-CIS



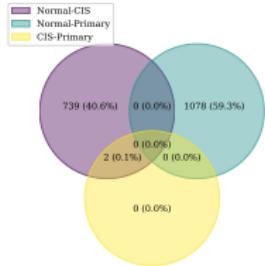
(b) Normal-Primary



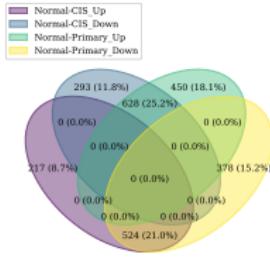
(c) CIS-Primary

Figure: DEG Volcano Plots with Recur samples in LUSC

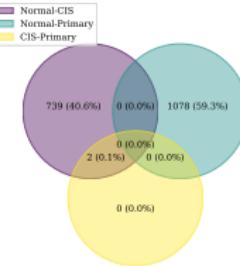
DEG Venn Diagram with Recur in LUSC



(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

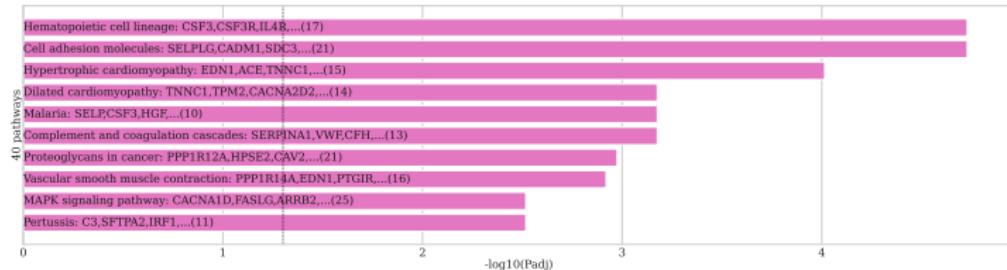


Figure: Up-regulated Pathways on Normal vs. CIS for Recur in LUSC

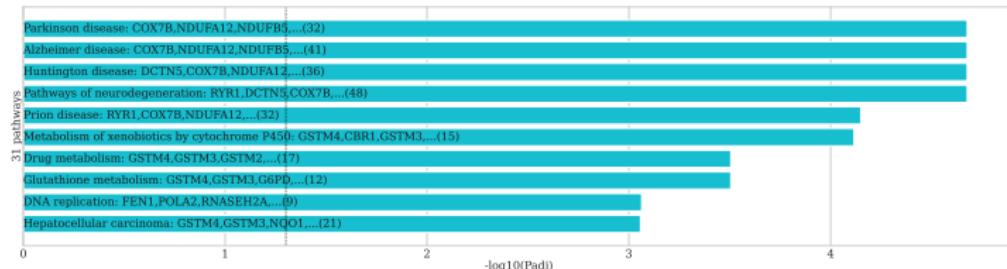


Figure: Down-regulated Pathways on Normal vs. CIS for Recur in LUSC

Enrichment test with Normal vs. Primary for Recur

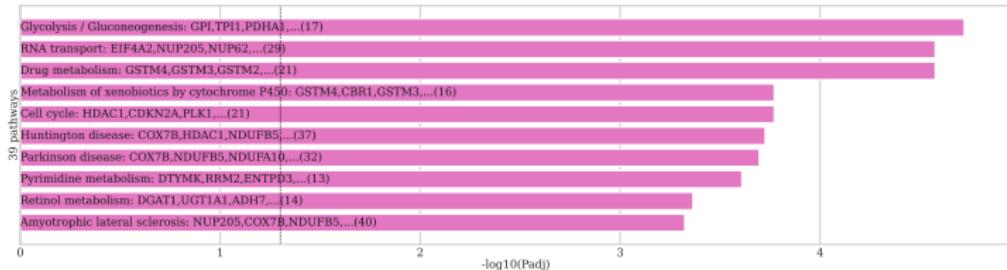


Figure: Up-regulated Pathways on Normal vs. Primary for Recur in LUSC

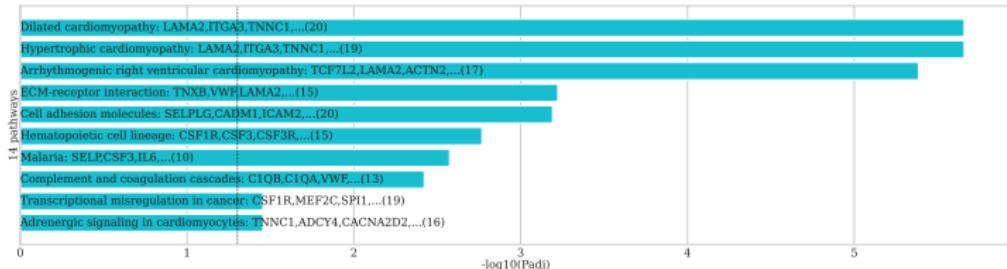


Figure: Down-regulated Pathways on Normal vs. Primary for Recur in LUSC

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)

Finding in Comparing within Recur in LUSC II

FAM107A

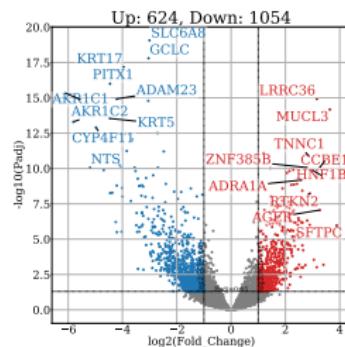
- ① Up-regulated in CIS, but down-regulated in Primary.
- ② May play a role in tumor development (L. Wang et al., 2000)
- ③ Negatively regulates focal adhesion assembly (Le et al., 2010)

4. Results

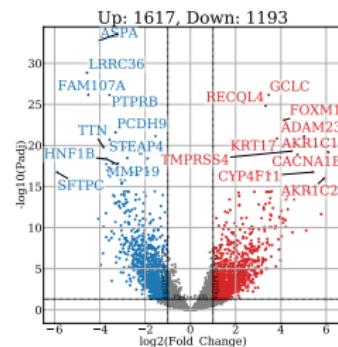
4.11. Differences in Gene Expression Levels

4.11.5. Within Non-recur in LUSC

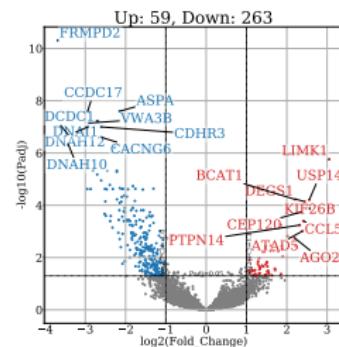
DEG Volcano Plots with Non-recr in LUSC



(a) Normal-CIS



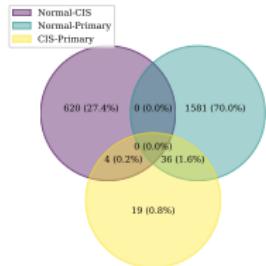
(b) Normal-Primary



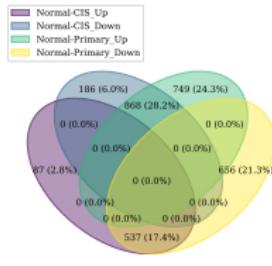
(c) CIS-Primary

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

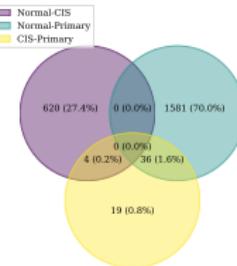
DEG Venn Diagram with Non-recur in LUSC



(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

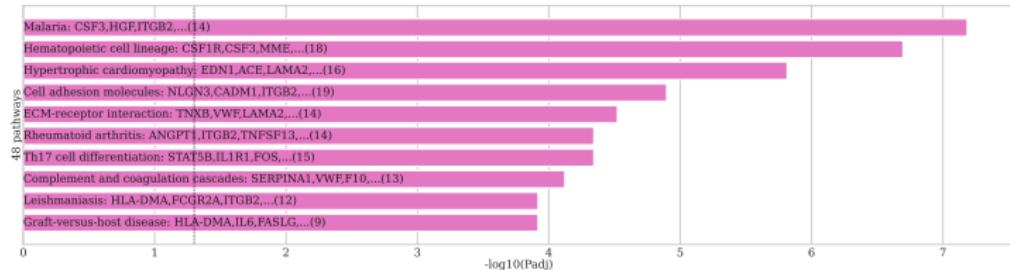


Figure: Up-regulated Pathways on Normal vs. CIS for Non-recur in LUSC

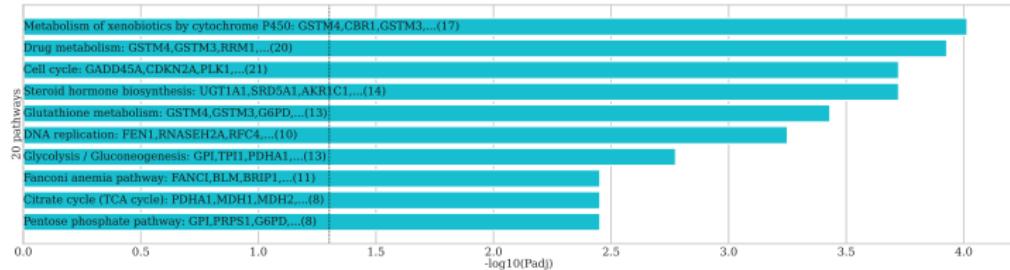


Figure: Down-regulated Pathways on Normal vs. CIS for Non-recur in LUSC

Enrichment test with Normal vs. Primary for Non-recur

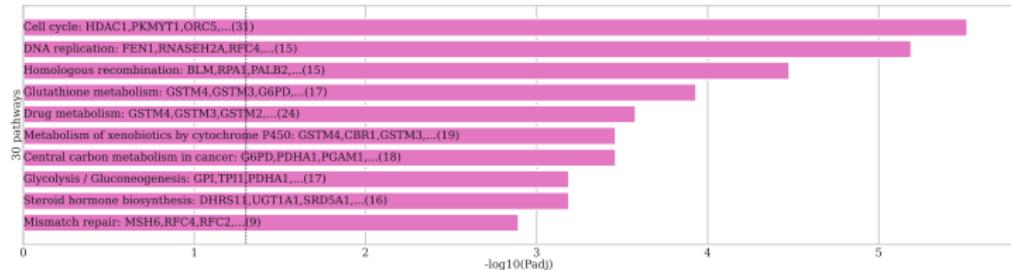


Figure: Up-regulated Pathways on Normal vs. Primary for Non-recur in LUSC

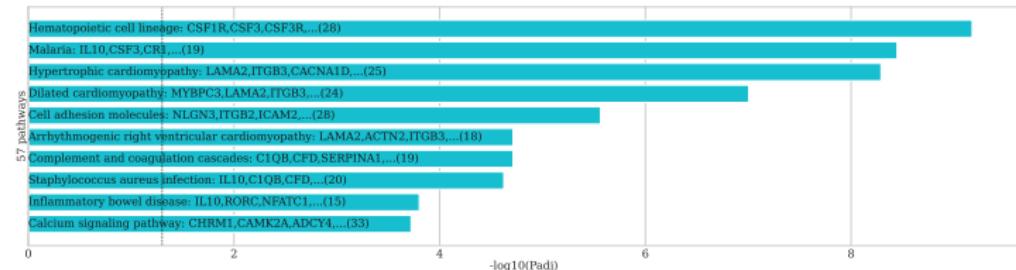


Figure: Down-regulated Pathways on Normal vs. Primary for Non-recur in LUSC

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

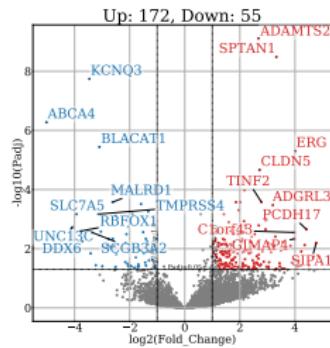
4.11.6. Within Non-recur in LUAD

LUAD Data Composition

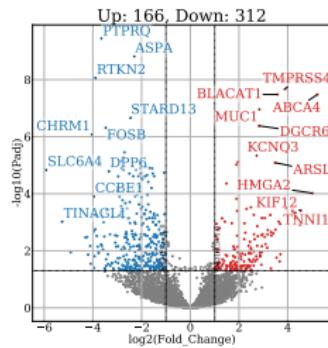
Table: Number of WTS LUAD samples

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

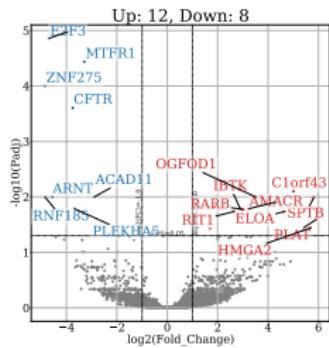
DEG Volcano Plots with Non-recr in LUAD



(a) Normal-AIS



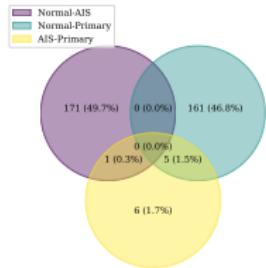
(b) Normal-Primary



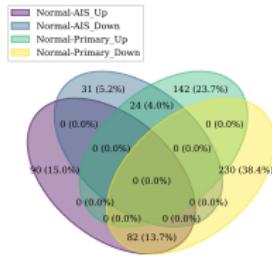
(c) AIS-Primary

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

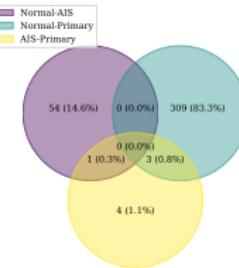
DEG Venn Diagram with Non-recur in LUAD



(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

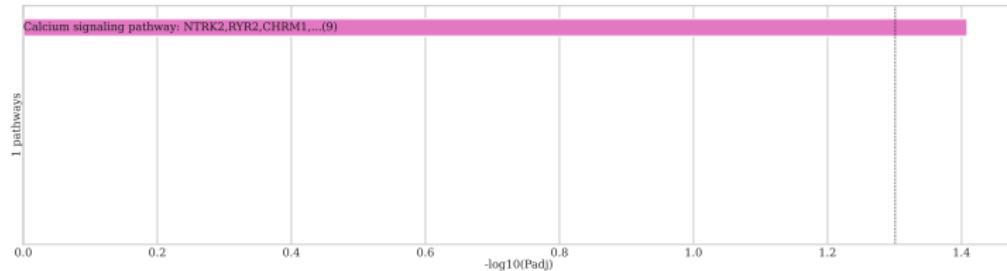


Figure: Up-regulated Pathways on Normal vs. AIS for Non-recur in LUAD

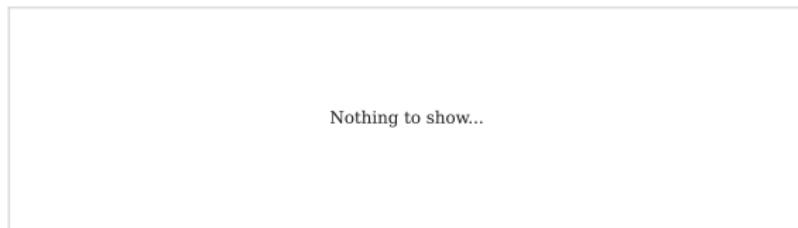


Figure: Down-regulated Pathways on Normal vs. AIS for Non-recur in LUAD

Enrichment test with Normal vs. Primary in LUAD

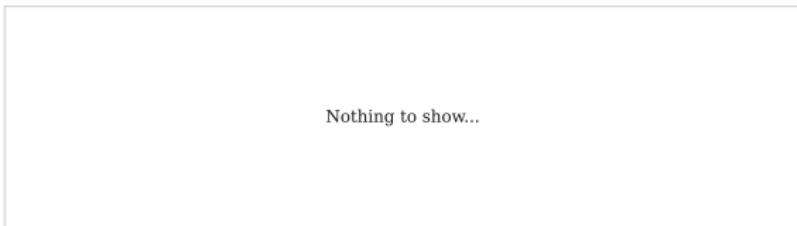


Figure: Up-regulated Pathways on Normal vs. Primary for Non-recur in LUAD

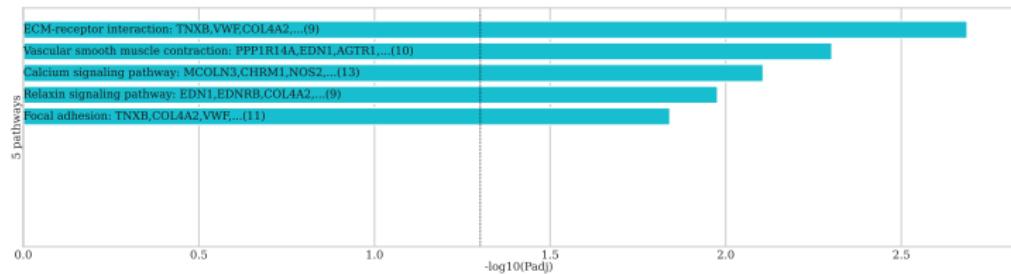


Figure: Down-regulated Pathways on Normal vs. Primary for Non-recur in LUAD

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. Mutation Shared Proportion

Mutation Shared Proportion?

Mutation Shared Proportion

Mutation Shared Proportion = $(\text{Precancer} \cap \text{Primary}) / \text{Primary}$

Selection Strategy

- ① Non-synonymous: point mutation, frame-shift, splice site, etc.
- ② Same site: chromosome, start position, & end position
- ③ Exact reference allele
- ④ Exact tumor allele

4. Results

4.12. Mutation Shared Proportion

4.12.1. BWA

Mutation Shared Proportion Distribution

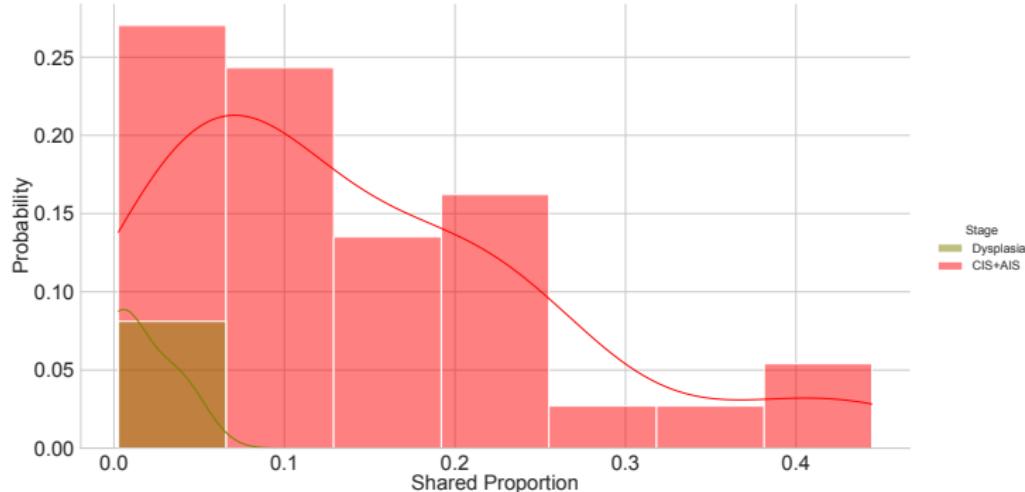
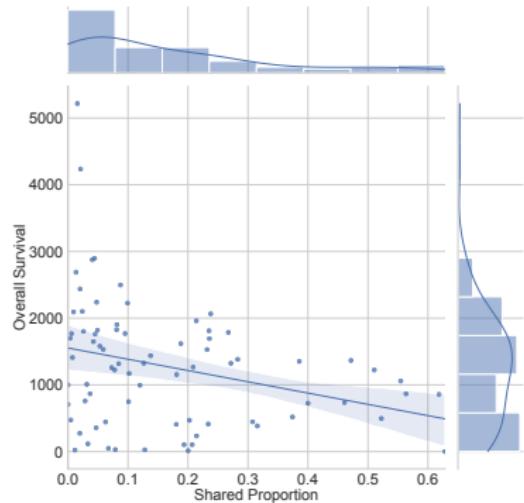
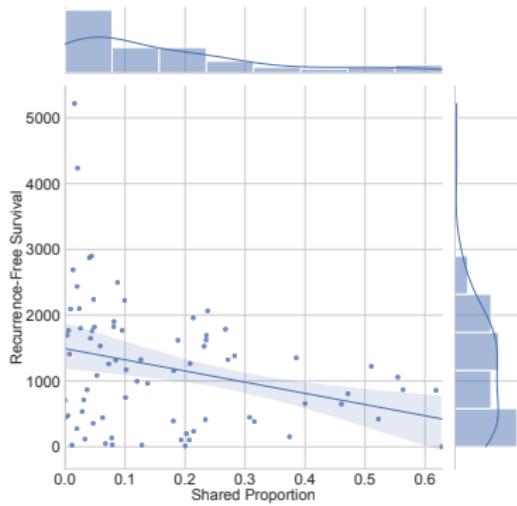


Figure: Mutation Shared Proportion Distribution in LUSC

Mutation Shared Proportion with Clinical Data I



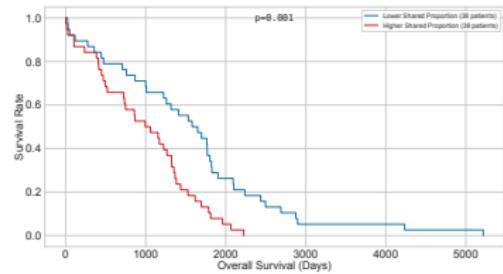
(a) Overall Survival



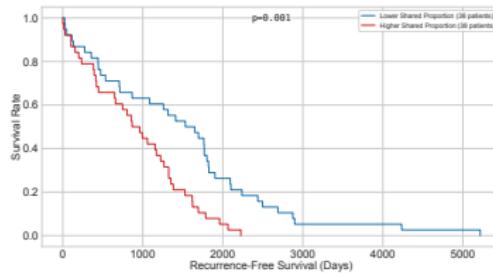
(b) Recurrence-Free Survival

Figure: Mutation Shared Proportion with Clinical Data from LUSC

Mutation Shared Proportion with Clinical Data II



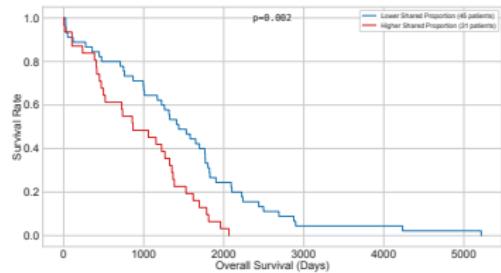
(a) Overall Survival



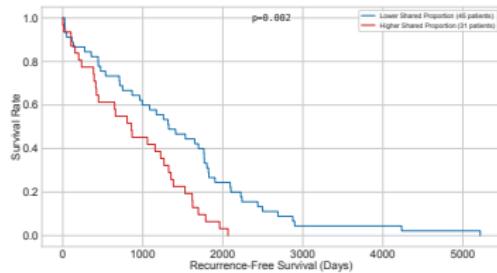
(b) Recurrence-Free Survival

Figure: K-M Survival Plot with Median separation from LUSC

Mutation Shared Proportion with Clinical Data III



(a) Overall Survival



(b) Recurrence-Free Survival

Figure: K-M Survival Plot with Mean separation from LUSC

Mutation Shared Genes

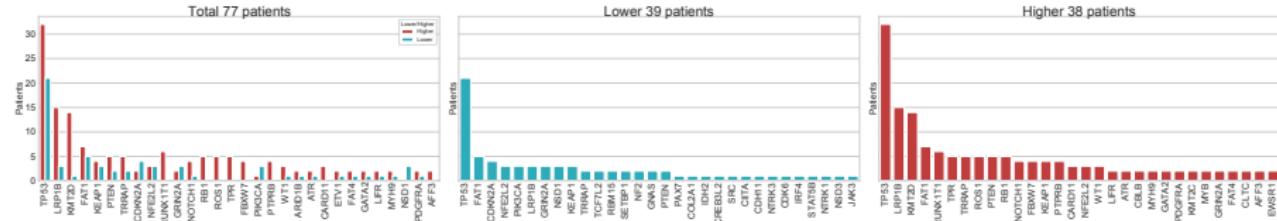


Figure: Mutation Shared Genes with Median separation from LUSC

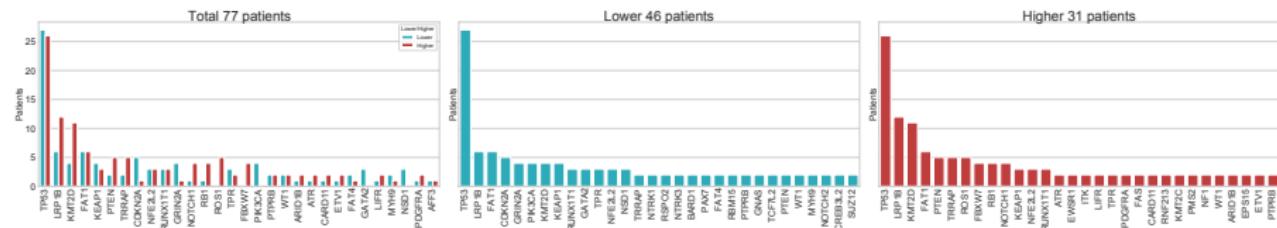


Figure: Mutation Shared Genes with Mean separation from LUSC

Mutation Shared Genes – Exact test I

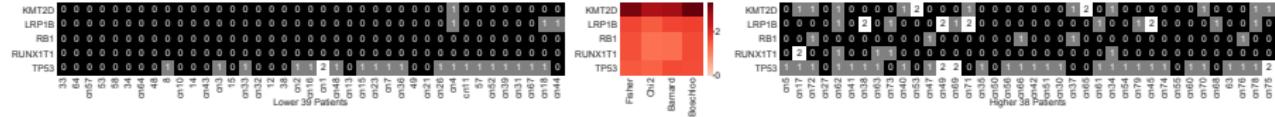


Figure: Exact Test on Mutation Shared Genes with Median separation from LUSC

Notable genes

- ① KMT2D
- ② LRP1B
- ③ RB1
- ④ RUNX1T1
- ⑤ TP53

KMT2D

- ① Lysine Methyltransferase 2D
- ② KMT2D have a role in chromatin remodeling
⇒ transcription & DNA repair (Shinsky, Monteith, Viggiano, & Cosgrove, 2015; Cho et al., 2007)
- ③ KMT2D deficiency impairs super-enhancers
∴ To confer a glycolytic vulnerability (Alam et al., 2020)
- ④ KMT2D mutation is associated with poor prognosis in NSCLC
(Ardeshir-Larijani et al., 2018)

LRP1B

- ① LDL Receptor Related Protein 1B
- ② LRP1B mutation is associated with favorable outcome to ICB across multiple cancer types (Brown et al., 2021)
- ③ LRP1B mutation is associated with tumor mutational burden in Lung cancer (Lan et al., 2019), especially NSCLC (H. Chen et al., 2019)
- ④ Higher prevalence of LRP1B mutation in LUAD with COPD (D. Xiao et al., 2017)

RB1

- ① Retinoblastoma Transcriptional Co-repressor 1
- ② Tumor suppressor ⇔ G1/S transition of cell cycle (Harbour, Luo, Dei Santi, Postigo, & Dean, 1999)
- ③ Common RB1 re-arrangements associated with histopathologic transformation
in non-smoking-related lung cancer (Pros et al., 2020)
- ④ Nicotine
 - ⇒ up-regulates FGFR3 & RB1 expression
 - ⇒ promote NSCLC cell proliferation & EMT transition (Du, Qi, Lu, Li, & Han, 2018)
- ⑤ RB1 mutation predicts poor outcomes in NSCLC (Bhateja et al., 2019)

RUNX1T1

- ① RUNX1 Partner Transcriptional Co-Repressor 1
- ② RUNX1T1 facilitates transcriptional repression
 ⇐ via DNA-binding transcription factors & histone-modifying enzymes (Davis, McGhee, & Meyers, 2003; Rossetti, Hoogeveen, & Sacchi, 2004; Melnick et al., 2000)
- ③ RUNX1T1 has a role in SCLC (He et al., 2021)
- ④ RUNX1T1 is associated with the brain metastatic process in lung cancer (Tomasini et al., 2020)

TP53

- ① Tumor Protein P53
- ② TP53 mutation is associated with response & longer survival under ICB in NSCLC (Assoun et al., 2019)
- ③ TP53 interacts with human lung cancer microbiome (Greathouse et al., 2018)

Mutation Shared Genes – Exact test VII

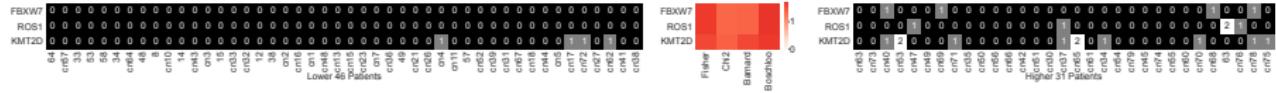


Figure: Exact Test on Mutation Shared Genes with Mean separation from LUSC

Notable genes

- ① FBXW7
- ② ROS1
- ③ KMT2D

Mutation Shared Genes – Exact test VIII

FBXW7

- ① F-Box and WD Repeat Domain Containing 7
- ② A part of E3 ubiquitin-protein ligase complex
⇒ mediates the ubiquitination and subsequent proteasomal degradation (Duda et al., 2012; Hao, Oehlmann, Sowa, Harper, & Pavletich, 2007; Yalla et al., 2018)
- ③ FBXW7 is a critical tumor suppressor of human cancers, including NSCLC (Yeh, Bellon, & Nicot, 2018)
- ④ FBXW7 mediates chemotherapeutic sensitivity & prognosis in NSCLC (Yokobori et al., 2014)
- ⑤ FBXW7-mediated ERK3 degradation proliferates lung cancer cells (An et al., 2022)

ROS1

- ① ROS Proto-Oncogene 1
- ② Crizotinib treatment in ROS1-rearranged NSCLC (Shaw et al., 2014)
- ③ Gene fusion (RET, ROS1, & ALK) in lung cancer (Takeuchi et al., 2012)
- ④ Resistance mechanisms of ROS1-positive lung cancer

4. Results

4.12. Mutation Shared Proportion

4.12.2. Bowtie2

Mutation Shared Proportion Distribution

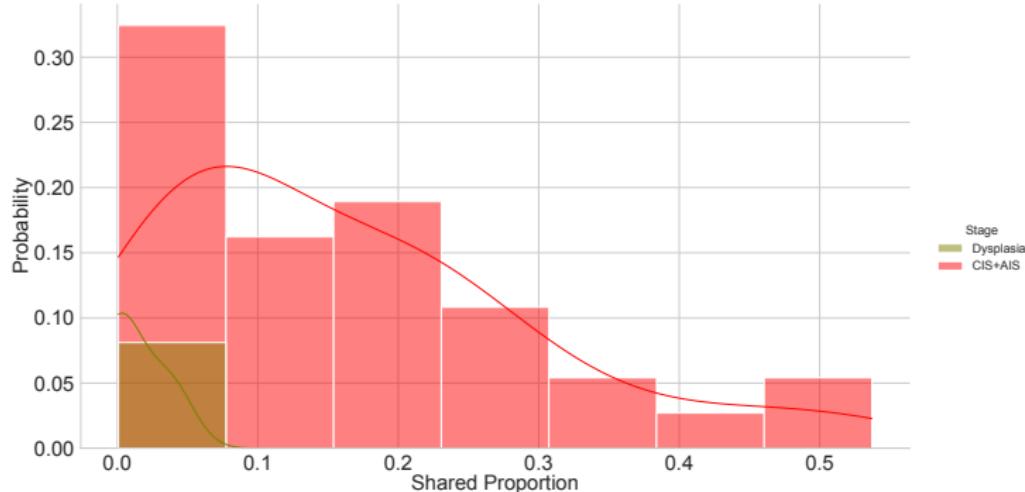
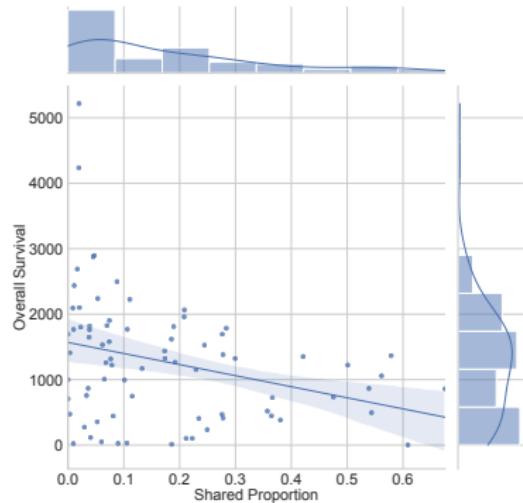
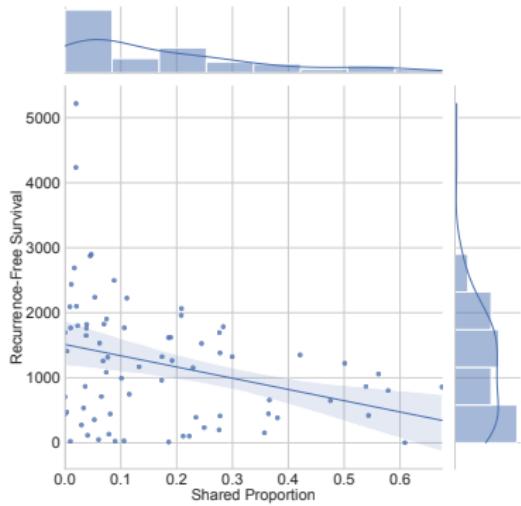


Figure: Mutation Shared Proportion Distribution in LUSC

Mutation Shared Proportion with Clinical Data I



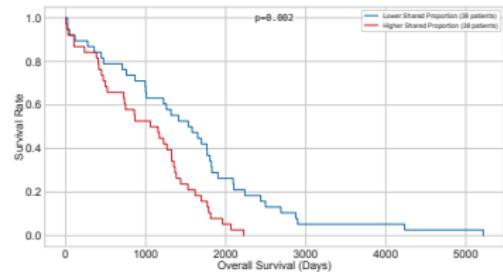
(a) Overall Survival



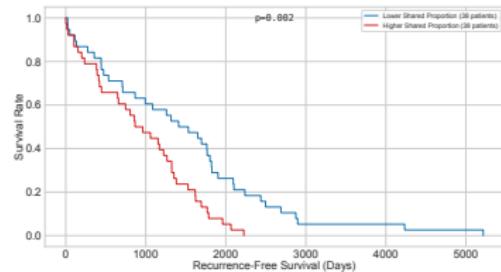
(b) Recurrence-Free Survival

Figure: Mutation Shared Proportion with Clinical Data from LUSC

Mutation Shared Proportion with Clinical Data II



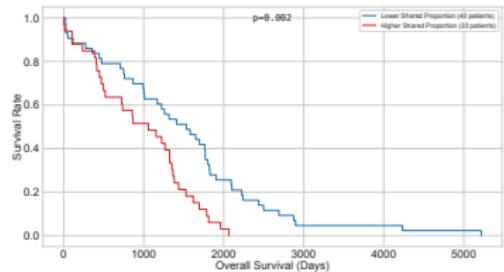
(a) Overall Survival



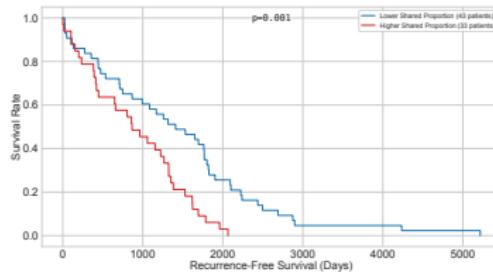
(b) Recurrence-Free Survival

Figure: K-M Survival Plot with Median separation from LUSC

Mutation Shared Proportion with Clinical Data III



(a) Overall Survival



(b) Recurrence-Free Survival

Figure: K-M Survival Plot with Mean separation from LUSC

Mutation Shared Genes

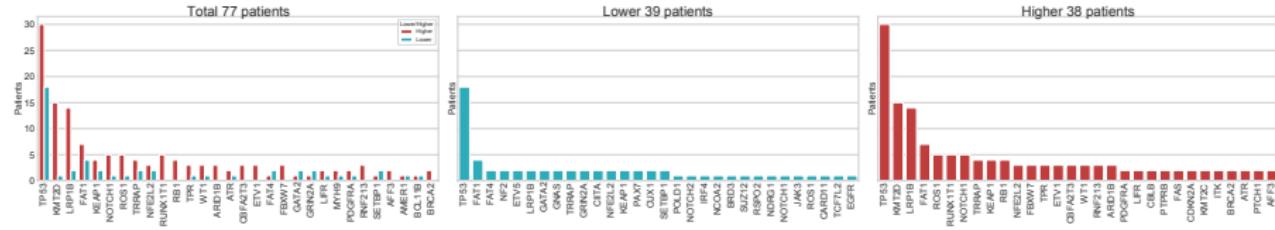


Figure: Mutation Shared Genes with Median separation from LUSC

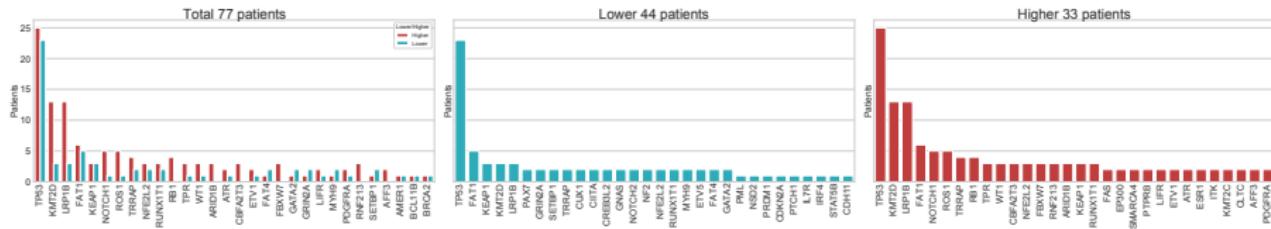


Figure: Mutation Shared Genes with Mean separation from LUSC

Mutation Shared Genes – Exact test I

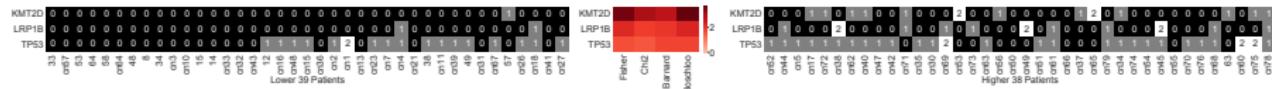
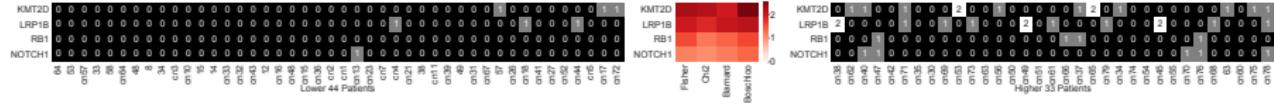


Figure: Exact Test on Mutation Shared Genes with Median separation from LUSC

Notable genes

- ① KMT2D
 - ② LRP1B
 - ③ TP53

Mutation Shared Genes – Exact test II



NOTCH1

- ① Neurogenic locus notch homolog protein 1
- ② A receptor for membrane-bound ligands to regulate cell-fate determination (Brütsch et al., 2010)
- ③ NOTCH1 controls cell proliferation, apoptosis, & differentiation in lung carcinoma (Wael et al., 2014)
- ④ NOTCH1 enhances ↑ EMT transition in lung cancer cell (Xie et al., 2012)
- ⑤ NOTCH1 mutation increased in lung cancer patient plasma (Liao et al., 2019)

Findings in Mutation Sharing Proportion

4. Results

4.13. Mutation Shared Proportion vs. Mutation Signature

title

4. Results

4.14. Identification of Microbial Sequences

PathSeq?

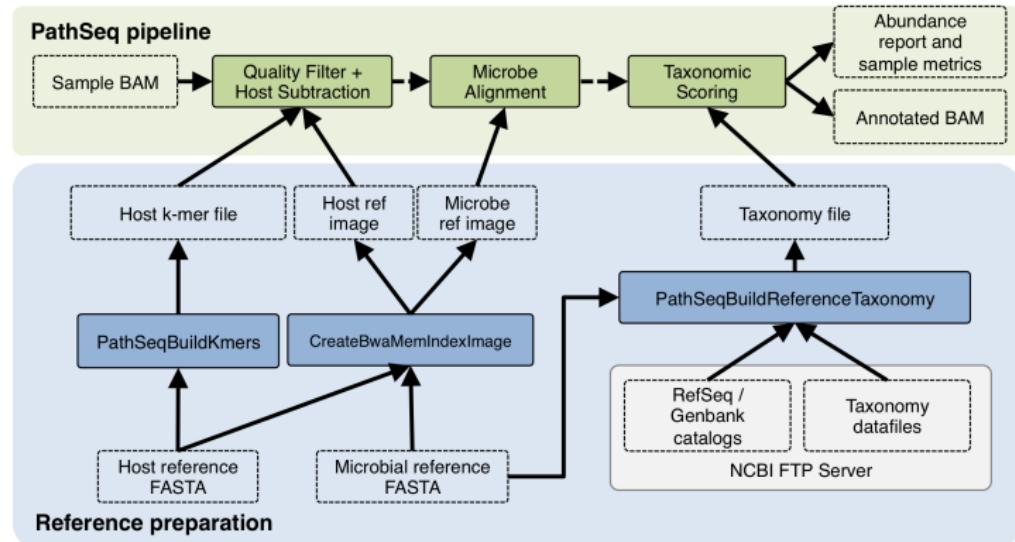


Figure: PathSeq Pipeline Diagram (Kostic et al., 2011; Walker et al., 2018)

4. Results

4.14. Identification of Microbial Sequences

4.14.1. WES – BWA

Taxonomy Distribution

4. Results

4.14. Identification of Microbial Sequences

4.14.2. WTS – STAR

Taxonomy Distribution

Diversity Indices

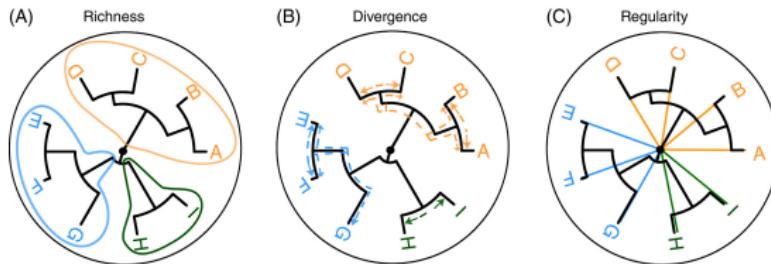


Figure: Three dimensions of phylogenetic information (Tucker et al., 2017)

- A quantitative measure that shows richness, divergence, and regularity (Tucker et al., 2017)
- Alpha diversity indices: the richness of taxa **at a single community**
- Beta diversity indices: the taxonomic differentiation **between communities**

Findings in Identification of Microbial Sequences

4. Results

4.15. Discovery of Gene Fusion

Arriba?

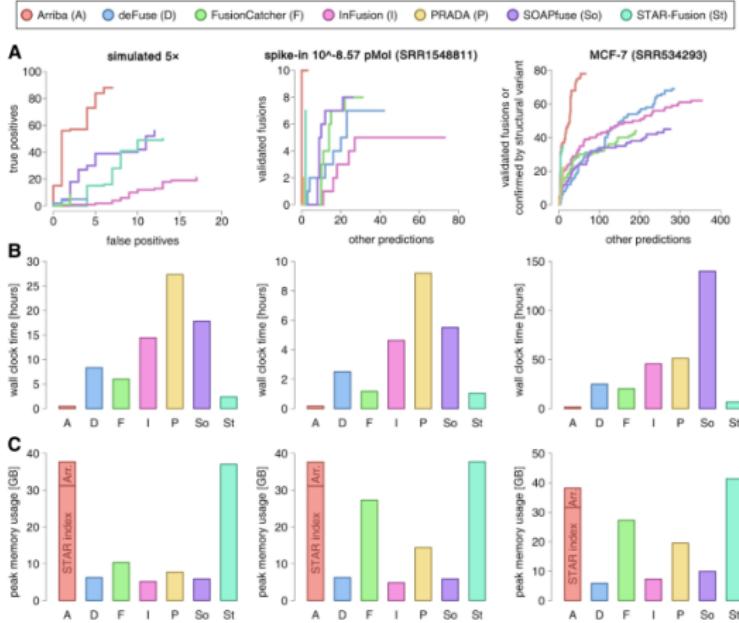


Figure: Benchmark of Arriba versus alternative methods (Uhrig et al., 2021)

Findings in Gene Fusion Discovery

5. Discussion

6. References

References I

- Aktaş, O. N., Öztürk, A. B., Erman, B., Erus, S., Tanju, S., & Dilege, S. (2018). Role of natural killer cells in lung cancer. *Journal of cancer research and clinical oncology*, 144(6), 997–1003.
- Alam, H., Tang, M., Maitituoheti, M., Dhar, S. S., Kumar, M., Han, C. Y., ... others (2020). Kmt2d deficiency impairs super-enhancers to confer a glycolytic vulnerability in lung cancer. *Cancer cell*, 37(4), 599–617.
- Alexandrov, L. B., Kim, J., Haradhvala, N. J., Huang, M. N., Ng, A. W. T., Wu, Y., ... others (2020). The repertoire of mutational signatures in human cancer. *Nature*, 578(7793), 94–101.
- Alexandrov, L. B., Nik-Zainal, S., Wedge, D. C., Aparicio, S. A., Behjati, S., Biankin, A. V., ... others (2013). Signatures of mutational processes in human cancer. *Nature*, 500(7463), 415–421.

References II

- Alnabulsi, A., Swan, R., Cash, B., Alnabulsi, A., & Murray, G. I. (2017). The differential expression of omega-3 and omega-6 fatty acid metabolising enzymes in colorectal cancer and its prognostic significance. *British journal of cancer*, 116(12), 1612–1620.
- An, H.-J., Lee, C.-J., Lee, G.-E., Choi, Y., Jeung, D., Chen, W., ... others (2022). Fbxw7-mediated erk3 degradation regulates the proliferation of lung cancer cells. *Experimental & molecular medicine*, 54(1), 35–46.
- Andrews, S., Krueger, F., Segonds-Pichon, A., Biggins, L., Krueger, C., & Wingett, S. (2012, January). *FastQC*. Babraham Institute. Babraham, UK.
- Ardeshir-Larijani, F., Bhateja, P., Lipka, M. B., Sharma, N., Fu, P., & Dowlati, A. (2018). Kmt2d mutation is associated with poor prognosis in non-small-cell lung cancer. *Clinical lung cancer*, 19(4), e489–e501.

References III

- Assoun, S., Theou-Anton, N., Nguenang, M., Cazes, A., Danel, C., Abbar, B., ... others (2019). Association of tp53 mutations with response and longer survival under immune checkpoint inhibitors in advanced non-small-cell lung cancer. *Lung cancer*, 132, 65–71.
- Behrend, S. J., Giotopoulou, G. A., Spella, M., & Stathopoulos, G. T. (2021). A role for club cells in smoking-associated lung adenocarcinoma. *European Respiratory Review*, 30(162).
- Bergstrom, E. N., Barnes, M., Martincorena, I., & Alexandrov, L. B. (2020). Generating realistic null hypothesis of cancer mutational landscapes using sigprofilersimulator. *BMC bioinformatics*, 21(1), 1–10.
- Bergstrom, E. N., Huang, M. N., Mahto, U., Barnes, M., Stratton, M. R., Rozen, S. G., & Alexandrov, L. B. (2019). Sigprofilermatrixgenerator: a tool for visualizing and exploring patterns of small mutational events. *BMC genomics*, 20(1), 1–12.

References IV

- Bernimoulin, M. P., Zeng, X.-L., Abbal, C., Giraud, S., Martinez, M., Michelin, O., ... Spertini, O. (2003). Molecular basis of leukocyte rolling on psgl-1: predominant role of core-2 o-glycans and of tyrosine sulfate residue 51. *Journal of Biological Chemistry*, 278(1), 37–47.
- Beyer, M., & Schultze, J. L. (2006). Regulatory t cells in cancer. *Blood*, 108(3), 804–811.
- Bhateja, P., Chiu, M., Wildey, G., Lipka, M. B., Fu, P., Yang, M. C. L., ... Dowlati, A. (2019). Retinoblastoma mutation predicts poor outcomes in advanced non small cell lung cancer. *Cancer medicine*, 8(4), 1459–1466.
- Bollig-Fischer, A., Bao, B., Manning, M., Dyson, G., Michelhaugh, S. K., Mittal, S., ... Mamdani, H. (2021). Role of novel cancer gene slitrk3 to activate ntrk3 in squamous cell lung cancer. *Molecular Biomedicine*, 2(1), 1–12.

References V

- Brahimi-Horn, M. C., Chiche, J., & Pouysségur, J. (2007). Hypoxia and cancer. *Journal of molecular medicine*, 85(12), 1301–1307.
- Brasch, F., Griese, M., Tredano, M., Johnen, G., Ochs, M., Rieger, C., ... Beers, M. (2004). Interstitial lung disease in a baby with a de novo mutation in the sftpc gene. *European Respiratory Journal*, 24(1), 30–39.
- Brown, L. C., Tucker, M. D., Sedhom, R., Schwartz, E. B., Zhu, J., Kao, C., ... others (2021). Lrp1b mutations are associated with favorable outcomes to immune checkpoint inhibitors across multiple cancer types. *Journal for immunotherapy of cancer*, 9(3).
- Brütsch, R., Liebler, S. S., Wüstehube, J., Bartol, A., Herberich, S. E., Adam, M. G., ... Fischer, A. (2010). Integrin cytoplasmic domain–associated protein-1 attenuates sprouting angiogenesis. *Circulation research*, 107(5), 592–601.

References VI

- Budanov, A. V., & Karin, M. (2008). p53 target genes sestrin1 and sestrin2 connect genotoxic stress and mtor signaling. *Cell*, 134(3), 451–460.
- Cailhier, J. F., Sawatzky, D. A., Kipari, T., Houlberg, K., Walbaum, D., Watson, S., ... others (2006). Resident pleural macrophages are key orchestrators of neutrophil recruitment in pleural inflammation. *American journal of respiratory and critical care medicine*, 173(5), 540–547.
- Cal, S., Freije, J. M., López, J. M., Takada, Y., & Lopez-Otin, C. (2000). Adam 23/mdc3, a human disintegrin that promotes cell adhesion via interaction with the $\alpha v \beta 3$ integrin through an rgd-independent mechanism. *Molecular biology of the cell*, 11(4), 1457–1469.

References VII

- Chantranupong, L., Wolfson, R. L., Orozco, J. M., Saxton, R. A., Scaria, S. M., Bar-Peled, L., ... Sabatini, D. M. (2014). The sestrins interact with gator2 to negatively regulate the amino-acid-sensing pathway upstream of mtorc1. *Cell reports*, 9(1), 1–8.
- Chen, E. Y., Tan, C. M., Kou, Y., Duan, Q., Wang, Z., Meirelles, G. V., ... Ma'ayan, A. (2013). Enrichr: interactive and collaborative html5 gene list enrichment analysis tool. *BMC bioinformatics*, 14(1), 1–14.
- Chen, H., Chong, W., Wu, Q., Yao, Y., Mao, M., & Wang, X. (2019). Association of Irp1b mutation with tumor mutation burden and outcomes in melanoma and non-small cell lung cancer patients treated with immune check-point blockades. *Frontiers in immunology*, 10, 1113.

References VIII

- Chen, J., Song, J., Yuan, P., Tian, Q., Ji, Y., Ren-Patterson, R., ... Weinberger, D. R. (2011). Orientation and cellular distribution of membrane-bound catechol-o-methyltransferase in cortical neurons: implications for drug development. *Journal of biological chemistry*, 286(40), 34752–34760.
- Chen, J.-M., Férec, C., & Cooper, D. N. (2013). Patterns and mutational signatures of tandem base substitutions causing human inherited disease. *Human mutation*, 34(8), 1119–1130.
- Chen, W., Hang, Y., Xu, W., Wu, J., Chen, L., Chen, J., ... Wang, H. (2019). Blacat1 predicts poor prognosis and serves as oncogenic lncrna in small-cell lung cancer. *Journal of cellular biochemistry*, 120(2), 2540–2546.

References IX

- Cho, Y.-W., Hong, T., Hong, S., Guo, H., Yu, H., Kim, D., ... others (2007). Ptip associates with mll3-and mll4-containing histone h3 lysine 4 methyltransferase complex. *Journal of Biological Chemistry*, 282(28), 20395–20406.
- Choi, B., Han, T.-S., Min, J., Hur, K., Lee, S.-M., Lee, H.-J., ... Yang, H.-K. (2017). Mal and tmem220 are novel dna methylation markers in human gastric cancer. *Biomarkers*, 22(1), 35–44.
- Christensen, I. B., Wu, Q., Bohlbro, A. S., Skals, M. G., Damkier, H. H., Hübner, C. A., ... Praetorius, J. (2020). Genetic disruption of slc4a10 alters the capacity for cellular metabolism and vectorial ion transport in the choroid plexus epithelium. *Fluids and Barriers of the CNS*, 17(1), 1–18.
- Collins, L. G., Haines, C., Perkel, R., & Enck, R. E. (2007). Lung cancer: diagnosis and management. *American family physician*, 75(1), 56–63.

References X

- Cordani, M., Butera, G., Dando, I., Torrens-Mas, M., Butturini, E., Pacchiana, R., ... others (2018). Mutant p53 blocks sesn1/ampk/pgc-1 α /ucp2 axis increasing mitochondrial O_2^- production in cancer cells. *British journal of cancer*, 119(8), 994–1008.
- Counts, S. E., He, B., Che, S., Ikonomovic, M. D., DeKosky, S. T., Ginsberg, S. D., & Mufson, E. J. (2007). $\alpha 7$ nicotinic receptor up-regulation in cholinergic basal forebrain neurons in alzheimer disease. *Archives of neurology*, 64(12), 1771–1776.
- Crowdis, J., He, M. X., Reardon, B., & Van Allen, E. M. (2020). Comut: visualizing integrated molecular information with comutation plots. *Bioinformatics*, 36(15), 4348–4349.
- Davis, J. N., McGhee, L., & Meyers, S. (2003). The eto (mtg8) gene family. *Gene*, 303, 1–10.

References XI

- Dawling, S., Roodi, N., Mernaugh, R. L., Wang, X., & Parl, F. F. (2001). Catechol-o-methyltransferase (comt)-mediated metabolism of catechol estrogens: comparison of wild-type and variant comt isoforms. *Cancer research*, 61(18), 6716–6722.
- Dean, B., & Scarr, E. (2021). Changes in cortical gene expression in the muscarinic m1 receptor knockout mouse: potential relevance to schizophrenia, alzheimer's disease and cognition. *npj Schizophrenia*, 7(1), 1–7.
- Deckers, J., Hammad, H., & Hoste, E. (2018). Langerhans cells: sensing the environment in health and disease. *Frontiers in immunology*, 9, 93.

References XII

- de Marco, M. C., Martín-Belmonte, F., Kremer, L., Albar, J. P., Correas, I., Vaerman, J. P., ... Alonso, M. A. (2002). Mal2, a novel raft protein of the mal family, is an essential component of the machinery for transcytosis in hepatoma hepg2 cells. *Journal of Cell Biology*, 159(1), 37–44.
- DePristo, M. A., Banks, E., Poplin, R., Garimella, K. V., Maguire, J. R., Hartl, C., ... others (2011). A framework for variation discovery and genotyping using next-generation dna sequencing data. *Nature genetics*, 43(5), 491.
- Dhar, M., Sepkovic, D. W., Hirani, V., Magnusson, R. P., & Lasker, J. M. (2008). Omega oxidation of 3-hydroxy fatty acids by the human cyp4f gene subfamily enzyme cyp4f11. *Journal of lipid research*, 49(3), 612–624.

References XIII

- Ding, B., Haidurov, A., Chawla, A., Parmigiani, A., van de Kamp, G., Dalina, A., ... others (2019). p53-inducible sestrins might play opposite roles in the regulation of early and late stages of lung carcinogenesis. *Oncotarget*, 10(65), 6997.
- Ding, L., Ley, T. J., Larson, D. E., Miller, C. A., Koboldt, D. C., Welch, J. S., ... others (2012). Clonal evolution in relapsed acute myeloid leukaemia revealed by whole-genome sequencing. *Nature*, 481(7382), 506–510.
- Dosenbach, N. U., Fair, D. A., Miezin, F. M., Cohen, A. L., Wenger, K. K., Dosenbach, R. A., ... others (2007). Distinct brain networks for adaptive and stable task control in humans. *Proceedings of the National Academy of Sciences*, 104(26), 11073–11078.

References XIV

- Du, X., Qi, F., Lu, S., Li, Y., & Han, W. (2018). Nicotine upregulates fgfr3 and rb1 expression and promotes non-small cell lung cancer cell proliferation and epithelial-to-mesenchymal transition via downregulation of mir-99b and mir-192. *Biomedicine & Pharmacotherapy*, 101, 656–662.
- Duda, D. M., Olszewski, J. L., Tron, A. E., Hammel, M., Lambert, L. J., Waddell, M. B., ... Schulman, B. A. (2012). Structure of a glomulin-rbx1-cul1 complex: inhibition of a ring e3 ligase through masking of its e2-binding surface. *Molecular cell*, 47(3), 371–382.
- Duhig, E., Clarke, B., Yang, I. A., Fong, K. M., Hunter, L., Windsor, M., ... others (2014). Comprehensive molecular profiling of lung adenocarcinoma: the cancer genome atlas research network. *Nature*, 511(7511), 543–550.

References XV

- Edson, K. Z., Prasad, B., Unadkat, J. D., Suhara, Y., Okano, T., Guengerich, F. P., & Rettie, A. E. (2013). Cytochrome p450-dependent catabolism of vitamin k: ω -hydroxylation catalyzed by human cyp4f2 and cyp4f11. *Biochemistry*, 52(46), 8276–8285.
- Erfani, N., Mehrabadi, S. M., Ghayumi, M. A., Haghshenas, M. R., Mojtabaei, Z., Ghaderi, A., & Amani, D. (2012). Increase of regulatory t cells in metastatic stage and ctla-4 over expression in lymphocytes of patients with non-small cell lung cancer (nsclc). *Lung cancer*, 77(2), 306–311.
- Fisler, D. A., Sikaria, D., Yavorski, J. M., Tu, Y. N., & Blanck, G. (2018). Elucidating feed-forward apoptosis signatures in breast cancer datasets: Higher fos expression associated with a better outcome. *Oncology letters*, 16(2), 2757–2763.

References XVI

- Geng, Y., Deng, L., Su, D., Xiao, J., Ge, D., Bao, Y., & Jing, H. (2016). Identification of crucial micrornas and genes in hypoxia-induced human lung adenocarcinoma cells. *Oncotargets and therapy*, 9, 4605.
- Gershenfeld, H. K., Hershberger, R. J., Shows, T. B., & Weissman, I. L. (1988). Cloning and chromosomal assignment of a human cdna encoding a t cell-and natural killer cell-specific trypsin-like serine protease. *Proceedings of the National Academy of Sciences*, 85(4), 1184–1188.
- Goldstraw, P., Ball, D., Jett, J. R., Le Chevalier, T., Lim, E., Nicholson, A. G., & Shepherd, F. A. (2011). Non-small-cell lung cancer. *The Lancet*, 378(9804), 1727–1740.

References XVII

- Goveia, J., Rohlenova, K., Taverna, F., Treps, L., Conradi, L.-C., Pircher, A., ... others (2020). An integrated gene expression landscape profiling approach to identify lung tumor endothelial cell heterogeneity and angiogenic candidates. *Cancer cell*, 37(1), 21–36.
- Greathouse, K. L., White, J. R., Vargas, A. J., Bliskovsky, V. V., Beck, J. A., von Muhlinen, N., ... others (2018). Interaction between the microbiome and tp53 in human lung cancer. *Genome biology*, 19(1), 1–16.
- Greczmiel, U., Kräutler, N. J., Pedrioli, A., Bartsch, I., Agnellini, P., Bedenikovic, G., ... Oxenius, A. (2017). Sustained t follicular helper cell response is essential for control of chronic viral infection. *Science Immunology*, 2(18).
- Gridelli, C., Rossi, A., Carbone, D. P., Guarize, J., Karachaliou, N., Mok, T., ... Rosell, R. (2015). Non-small-cell lung cancer. *Nature reviews Disease primers*, 1(1), 1–16.

References XVIII

- Grzegrzolka, J., Olbromski, M., Gomulkiewicz, A., Piotrowska, A., Glatzel-Plucinska, N., Ratajczak, K., ... others (2021). Role of tesmin expression in non-small cell lung cancer. *Oncology Letters*, 21(1), 1–1.
- Gueguen, P., Metoikidou, C., Dupic, T., Lawand, M., Goudot, C., Baulande, S., ... others (2021). Contribution of resident and circulating precursors to tumor-infiltrating cd8+ t cell populations in lung cancer. *Science Immunology*, 6(55), eabd5778.
- Hameed, A., Lowrey, D., Lichtenheld, M., & Podack, E. (1988). Characterization of three serine esterases isolated from human il-2 activated killer cells. *The Journal of Immunology*, 141(9), 3142–3147.

References XIX

- Hao, B., Oehlmann, S., Sowa, M. E., Harper, J. W., & Pavletich, N. P. (2007). Structure of a fbw7-skp1-cyclin e complex: multisite-phosphorylated substrate recognition by scf ubiquitin ligases. *Molecular cell*, 26(1), 131–143.
- Harbour, J. W., Luo, R. X., Dei Santi, A., Postigo, A. A., & Dean, D. C. (1999). Cdk phosphorylation triggers sequential intramolecular interactions that progressively block rb functions as cells move through g1. *Cell*, 98(6), 859–869.
- He, T., Wildey, G., McColl, K., Savadelis, A., Spainhower, K., McColl, C., ... others (2021). Identification of runx1t1 as a potential epigenetic modifier in small-cell lung cancer. *Molecular Oncology*, 15(1), 195–209.

References XX

- Henderson, L. B., Melton, K., Wert, S., Couriel, J., Bush, A., Ashworth, M., & Nogee, L. M. (2013). Large abca3 and sftpc deletions resulting in lung disease. *Annals of the American Thoracic Society*, 10(6), 602–607.
- Hong, S., Won, Y.-J., Lee, J. J., Jung, K.-W., Kong, H.-J., Im, J.-S., ... others (2021). Cancer statistics in korea: Incidence, mortality, survival, and prevalence in 2018. *Cancer Research and Treatment: Official Journal of Korean Cancer Association*, 53(2), 301.
- Hong, Z., Chang, L.-L., Fang-Jie, Y., Yan, H., Chen-Ming, Z., Tian-Yi, Z., ... others (2018). Akr1c1 activates stat3 to promote the metastasis of non-small cell lung cancer. *Theranostics*, 8(3), 676.
- Hu, C., Lv, H., Pan, G., Cao, H., Deng, Z., Hu, C., ... Zhou, J. (2011). The expression of adam23 and its correlation with promoter methylation in non-small-cell lung carcinoma. *International journal of experimental pathology*, 92(5), 333–339.

References XXI

- Huang, F.-X., Chen, H.-J., Zheng, F.-X., Gao, Z.-Y., Sun, P.-F., Peng, Q., ... others (2019). Lncrna blacat1 is involved in chemoresistance of non-small cell lung cancer cells by regulating autophagy. *International journal of oncology*, 54(1), 339–347.
- Islam, S. M. A., Wu, Y., Díaz-Gay, M., Bergstrom, E. N., He, Y., Barnes, M., ... Alexandrov, L. B. (2021). Uncovering novel mutational signatures by de novo extraction with sigprofilerextractor. *bioRxiv*. Retrieved from <https://www.biorxiv.org/content/early/2021/05/16/2020.12.13.422570> doi: 10.1101/2020.12.13.422570
- Jew, B., Alvarez, M., Rahmani, E., Miao, Z., Ko, A., Garske, K. M., ... Halperin, E. (2020). Accurate estimation of cell composition in bulk expression through robust integration of single-cell information. *Nature communications*, 11(1), 1–11.

References XXII

- Jia, Q., Wu, W., Wang, Y., Alexander, P. B., Sun, C., Gong, Z., ... others (2018). Local mutational diversity drives intratumoral immune heterogeneity in non-small cell lung cancer. *Nature communications*, 9(1), 1–10.
- Jin, Y., Duan, L., Lee, S. H., Kloosterboer, H. J., Blair, I. A., & Penning, T. M. (2009). Human cytosolic hydroxysteroid dehydrogenases of the aldo-ketoreductase superfamily catalyze reduction of conjugated steroids. *Journal of Biological Chemistry*, 284(15), 10013–10022.
- Kalsotra, A., Turman, C. M., Kikuta, Y., & Strobel, H. W. (2004). Expression and characterization of human cytochrome p450 4f11: Putative role in the metabolism of therapeutic drugs and eicosanoids. *Toxicology and applied pharmacology*, 199(3), 295–304.
- Kanehisa, M., Furumichi, M., Sato, Y., Ishiguro-Watanabe, M., & Tanabe, M. (2021). Kegg: integrating viruses and cellular organisms. *Nucleic acids research*, 49(D1), D545–D551.

References XXIII

- Kim, E. Y., Cha, Y. J., Lee, S. H., Jeong, S., Choi, Y. J., Moon, D. H., ... Chang, Y. S. (2022). Early lung carcinogenesis and tumor microenvironment observed by single-cell transcriptome analysis. *Translational oncology*, 15(1), 101277.
- Kim, N., Kim, H. K., Lee, K., Hong, Y., Cho, J. H., Choi, J. W., ... others (2020). Single-cell rna sequencing demonstrates the molecular and cellular reprogramming of metastatic lung adenocarcinoma. *Nature communications*, 11(1), 1–15.
- Kostic, A. D., Ojesina, A. I., Pedamallu, C. S., Jung, J., Verhaak, R. G., Getz, G., & Meyerson, M. (2011). Pathseq: software to identify or discover microbes by deep sequencing of human tissue. *Nature biotechnology*, 29(5), 393–396.

References XXIV

- Kou, F., Sun, H., Wu, L., Li, B., Zhang, B., Wang, X., & Yang, L. (2020). Top2a promotes lung adenocarcinoma cells' malignant progression and predicts poor prognosis in lung adenocarcinoma. *Journal of Cancer*, 11(9), 2496.
- Krähenbühl, O., Rey, C., Jenne, D., Lanzavecchia, A., Groscurth, P., Carrel, S., & Tschopp, J. (1988). Characterization of granzymes a and b isolated from granules of cloned human cytotoxic t lymphocytes. *The Journal of Immunology*, 141(10), 3471–3477.
- Kuleshov, M. V., Jones, M. R., Rouillard, A. D., Fernandez, N. F., Duan, Q., Wang, Z., ... others (2016). Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. *Nucleic acids research*, 44(W1), W90–W97.

References XXV

- Kumari, S., Arora, M., Singh, J., Chauhan, S. S., Kumar, S., & Chopra, A. (2021). L-selectin expression is associated with inflammatory microenvironment and favourable prognosis in breast cancer. *3 Biotech*, 11(2), 1–13.
- Kyotani, Y., Takasawa, S., & Yoshizumi, M. (2019). Proliferative pathways of vascular smooth muscle cells in response to intermittent hypoxia. *International journal of molecular sciences*, 20(11), 2706.
- Lacal, P. M., Petrillo, M. G., Ruffini, F., Muzi, A., Bianchini, R., Ronchetti, S., ... Nocentini, G. (2013). Glucocorticoid-induced tumor necrosis factor receptor family-related ligand triggering upregulates vascular cell adhesion molecule-1 and intercellular adhesion molecule-1 and promotes leukocyte adhesion. *Journal of Pharmacology and Experimental Therapeutics*, 347(1), 164–172.

References XXVI

- Lan, S., Li, H., Liu, Y., Ma, L., Liu, X., Liu, Y., ... Cheng, Y. (2019). Somatic mutation of *Irp1b* is associated with tumor mutational burden in patients with lung cancer. *Lung cancer*, 132, 154–156.
- Lara-Lemus, R. (2019). On the role of myelin and lymphocyte protein (mal) in cancer: a puzzle with two faces. *Journal of Cancer*, 10(10), 2312.
- Le, P., Angers-Loustau, A., De Oliveira, R., Ajlan, A., Brassard, C., Dudley, A., ... others (2010). Drr drives brain cancer invasion by regulating cytoskeletal-focal adhesion dynamics. *Oncogene*, 29(33), 4636–4647.
- Lee, D., Lee, G. K., Yoon, K.-A., & Lee, J. S. (2013). Pathway-based analysis using genome-wide association data from a korean non-small cell lung cancer study. *PLoS one*, 8(6), e65396.

References XXVII

- Li, B., Brady, S. W., Ma, X., Shen, S., Zhang, Y., Li, Y., ... others (2020). Therapy-induced mutations drive the genomic landscape of relapsed acute lymphoblastic leukemia. *Blood*, 135(1), 41–55.
- Li, B., & Dewey, C. N. (2011). Rsem: accurate transcript quantification from rna-seq data with or without a reference genome. *BMC bioinformatics*, 12(1), 1–16.
- Li, B., Meng, Y.-Q., Li, Z., Yin, C., Lin, J.-P., Zhu, D.-J., & Zhang, S.-B. (2019). Mir-629-3p-induced downregulation of sftpc promotes cell proliferation and predicts poor survival in lung adenocarcinoma. *Artificial cells, nanomedicine, and biotechnology*, 47(1), 3286–3296.
- Li, D., Zhang, J., Wu, L., Yang, X., Chen, Z., & Yuan, J. (2021). Myelin and lymphocyte protein (mal): A novel biomarker for uterine corpus endometrial carcinoma. *Cancer Management and Research*, 13, 7311.

References XXVIII

- Liao, Y., Ma, Z., Zhang, Y., Li, D., Lv, D., Chen, Z., ... others (2019). Targeted deep sequencing from multiple sources demonstrates increased notch1 alterations in lung cancer patient plasma. *Cancer medicine*, 8(12), 5673–5686.
- Lin, C., Song, H., Huang, C., Yao, E., Gacayan, R., Xu, S.-M., & Chuang, P.-T. (2012). Alveolar type ii cells possess the capability of initiating lung tumor development. *PLoS one*, 7(12), e53817.
- Lin, Z., Thorenoor, N., Wu, R., DiAngelo, S. L., Ye, M., Thomas, N. J., ... Floros, J. (2018). Genetic association of pulmonary surfactant protein genes, sftpa1, sftpa2, sftpib, sftpc, and sftpd with cystic fibrosis. *Frontiers in immunology*, 9, 2256.
- Liu, X., Xu, X., Deng, W., Huang, M., Wu, Y., Zhou, Z., ... others (2019). Ccl18 enhances migration, invasion and emt by binding ccr8 in bladder cancer cells. *Molecular medicine reports*, 19(3), 1678–1686.

References XXIX

- Liu, Y.-Z., Wang, B.-S., Jiang, Y.-Y., Cao, J., Hao, J.-J., Zhang, Y., ... Wang, M.-R. (2017). Mcms expression in lung cancer: implication of prognostic significance. *Journal of Cancer*, 8(18), 3641.
- Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for rna-seq data with deseq2. *Genome biology*, 15(12), 1–21.
- Ma, G., Ji, D., Qu, X., Liu, S., Yang, X., Wang, G., ... Du, J. (2019). Mining and validating the expression pattern and prognostic value of acetylcholine receptors in non-small cell lung cancer. *Medicine*, 98(20).
- Ma, W., Wang, B., Zhang, Y., Wang, Z., Niu, D., Chen, S., ... others (2019). Prognostic significance of top2a in non-small cell lung cancer revealed by bioinformatic analysis. *Cancer cell international*, 19(1), 1–17.

References XXX

- Marcé-Grau, A., Elorza-Vidal, X., Pérez-Rius, C., Ruiz-Nel·lo, A., Sala-Coromina, J., Gabau, E., ... Macaya, A. (2021). Muscarinic acetylcholine receptor m1 mutations causing neurodevelopmental disorder and epilepsy. *Human Mutation*.
- Mason, R. J. (2006). Biology of alveolar type ii cells. *Respirology*, 11, S12–S15.
- Maugeri, A., Klevering, B. J., Rohrschneider, K., Blankenagel, A., Brunner, H. G., Deutman, A. F., ... Cremers, F. P. (2000). Mutations in the abca4 (abcr) gene are the major cause of autosomal recessive cone-rod dystrophy. *The American Journal of Human Genetics*, 67(4), 960–966.

References XXXI

- Melnick, A. M., Westendorf, J. J., Polinger, A., Carlile, G. W., Arai, S., Ball, H. J., ... Licht, J. D. (2000). The eto protein disrupted in t(8; 21)-associated acute myeloid leukemia is a corepressor for the promyelocytic leukemia zinc finger protein. *Molecular and cellular biology*, 20(6), 2075–2086.
- Mermel, C. H., Schumacher, S. E., Hill, B., Meyerson, M. L., Beroukhim, R., & Getz, G. (2011). Gistic2. 0 facilitates sensitive and confident localization of the targets of focal somatic copy-number alteration in human cancers. *Genome biology*, 12(4), 1–14.
- Minna, J. D., Roth, J. A., & Gazdar, A. F. (2002). Focus on lung cancer. *Cancer cell*, 1(1), 49–52.
- Modi, B. G., Neustadter, J., Binda, E., Lewis, J., Filler, R. B., Roberts, S. J., ... others (2012). Langerhans cells facilitate epithelial dna damage and squamous cell carcinoma. *Science*, 335(6064), 104–108.

References XXXII

- Mondejar-Parreño, G., Perez-Vizcaino, F., & Cogolludo, A. (2020). Kv7 channels in lung diseases. *Frontiers in Physiology*, 11, 634.
- Narayanan, S., Kawaguchi, T., Yan, L., Peng, X., Qi, Q., & Takabe, K. (2018). Cytolytic activity score to assess anticancer immunity in colorectal cancer. *Annals of surgical oncology*, 25(8), 2323–2331.
- Nikolaou, S., Qiu, S., Fiorentino, F., Simillis, C., Rasheed, S., Tekkis, P., & Kontovounisios, C. (2020). The role of neuropeptides and their receptors in non-gastrointestinal cancers: a review. *Cell Communication and Signaling*, 18(1), 1–10.
- Nik-Zainal, S., Alexandrov, L. B., Wedge, D. C., Van Loo, P., Greenman, C. D., Raine, K., ... others (2012). Mutational processes molding the genomes of 21 breast cancers. *Cell*, 149(5), 979–993.

References XXXIII

- Niu, Y., Lin, A., Luo, P., Zhu, W., Wei, T., Tang, R., ... Zhang, J. (2020). Prognosis of lung adenocarcinoma patients with ntrk3 mutations to immune checkpoint inhibitors. *Frontiers in pharmacology*, 11, 1213.
- Olingy, C. E., Dinh, H. Q., & Hedrick, C. C. (2019). Monocyte heterogeneity and functions in cancer. *Journal of leukocyte biology*, 106(2), 309–322.
- Ota, M., Mochizuki, S., Shimoda, M., Abe, H., Miyamae, Y., Ishii, K., ... Okada, Y. (2016). Adam 23 is downregulated in side population and suppresses lung metastasis of lung carcinoma cells. *Cancer science*, 107(4), 433–443.
- Paul, R., Hu, B., Musahl, C., Hameister, H., & Knippers, R. (1996). Coding sequence and chromosome mapping of the human gene (cdc46) for replication protein hcdc46/mcm5. *Cytogenetic and Genome Research*, 73(4), 317–321.

References XXXIV

- Penning, T. M., Burczynski, M. E., Jez, J. M., Hung, C.-F., Lin, H.-K., Ma, H., ... RATNAM, K. (2000). Human 3α -hydroxysteroid dehydrogenase isoforms (akr1c1–akr1c4) of the aldo-keto reductase superfamily: functional plasticity and tissue distribution reveals roles in the inactivation and formation of male and female sex hormones. *Biochemical journal*, 351(1), 67–77.
- Pinto, A., Rega, A., Crother, T. R., & Sorrentino, R. (2012). Plasmacytoid dendritic cells and their therapeutic activity in cancer. *Oncoimmunology*, 1(5), 726–734.
- Plitas, G., Konopacki, C., Wu, K., Bos, P. D., Morrow, M., Putintseva, E. V., ... Rudensky, A. Y. (2016). Regulatory t cells exhibit distinct features in human breast cancer. *Immunity*, 45(5), 1122–1134.
- Poonia, B., Ayithan, N., Nandi, M., Masur, H., & Kottilil, S. (2018a). Hbv induces inhibitory fcrl receptor on b cells and dysregulates b cell-t follicular helper cell axis. *Scientific reports*, 8(1), 1–14.

References XXXV

- Poonia, B., Ayithan, N., Nandi, M., Masur, H., & Kottilil, S. (2018b). Hbv induces inhibitory fcrl receptor on b cells and dysregulates b cell-t follicular helper cell axis. *Scientific reports*, 8(1), 1–14.
- Pros, E., Saigi, M., Alameda, D., Gomez-Mariano, G., Martinez-Delgado, B., Alburquerque-Bejar, J., ... others (2020). Genome-wide profiling of non-smoking-related lung cancer cells reveals common rb1 rearrangements associated with histopathologic transformation in egfr-mutant tumors. *Annals of Oncology*, 31(2), 274–282.
- Rawlins, E. L., & Hogan, B. L. (2008). Ciliated epithelial cell lifespan in the mouse trachea and lung. *American Journal of Physiology-Lung Cellular and Molecular Physiology*, 295(1), L231–L234.
- Riester, M., Singh, A. P., Brannon, A. R., Yu, K., Campbell, C. D., Chiang, D. Y., & Morrissey, M. P. (2016). Purecn: copy number calling and snv classification using targeted short read sequencing. *Source code for biology and medicine*, 11(1), 1–13.

References XXXVI

- Rokicki, W., Rokicki, M., Wojtacha, J., & Dżeljijli, A. (2016). The role and importance of club cells (clara cells) in the pathogenesis of some respiratory diseases. *Kardiochirurgia i torakochirurgia polska= Polish journal of cardio-thoracic surgery*, 13(1), 26.
- Rossetti, S., Hoogeveen, A. T., & Sacchi, N. (2004). The mtg proteins: chromatin repression players with a passion for networking. *Genomics*, 84(1), 1–9.
- Roth, A., Khattra, J., Yap, D., Wan, A., Laks, E., Biele, J., . . . Shah, S. P. (2014). Pyclone: statistical inference of clonal population structure in cancer. *Nature methods*, 11(4), 396–398.
- Salamon, P., Mekori, Y. A., & Shefler, I. (2020). Lung cancer-derived extracellular vesicles: a possible mediator of mast cell activation in the tumor microenvironment. *Cancer Immunology, Immunotherapy*, 69(3), 373–381.

References XXXVII

- Santiago, L., Castro, M., Sanz-Pamplona, R., Garzón, M., Ramirez-Labrada, A., Tapia, E., ... others (2020). Extracellular granzyme a promotes colorectal cancer development by enhancing gut inflammation. *Cell reports*, 32(1), 107847.
- Schroeder, B. C., Kubisch, C., Stein, V., & Jentsch, T. J. (1998). Moderate loss of function of cyclic-amp-modulated kcnq2/kcnq3 k⁺ channels causes epilepsy. *Nature*, 396(6712), 687–690.
- Shaw, A. T., Ou, S.-H. I., Bang, Y.-J., Camidge, D. R., Solomon, B. J., Salgia, R., ... others (2014). Crizotinib in ros1-rearranged non-small-cell lung cancer. *New England Journal of Medicine*, 371(21), 1963–1971.
- Shin, M. H., Kim, J., Lim, S. A., Kim, J., Kim, S.-J., & Lee, K.-M. (2020). Nk cell-based immunotherapies in cancer. *Immune network*, 20(2).

References XXXVIII

- Shinsky, S. A., Monteith, K. E., Viggiano, S., & Cosgrove, M. S. (2015). Biochemical reconstitution and phylogenetic comparison of human set1 family core complexes involved in histone methylation. *Journal of Biological Chemistry*, 290(10), 6361–6375.
- Sica, A., Allavena, P., & Mantovani, A. (2008). Cancer related inflammation: the macrophage connection. *Cancer letters*, 267(2), 204–215.
- Singh, N. A., Westenskow, P., Charlier, C., Pappas, C., Leslie, J., Dillon, J., ... Leppert, M. F. (2003). Kcnq2 and kcnq3 potassium channel genes in benign familial neonatal convulsions: expansion of the functional and mutation spectrum. *Brain*, 126(12), 2726–2737.
- Soltis, A. R., Dalgard, C. L., Pollard, H. B., & Wilkerson, M. D. (2020). Mutenricher: a flexible toolset for somatic mutation enrichment analysis of tumor whole genomes. *BMC bioinformatics*, 21(1), 1–8.

References XXXIX

- Spella, M., Lolis, I., Pepe, M. A., Chen, Y., Armaka, M., Lamort, A.-S., ... others (2019). Club cells form lung adenocarcinomas and maintain the alveoli of adult mice. *Elife*, 8, e45571.
- Surti, T. S., Huang, L., Jan, Y. N., Jan, L. Y., & Cooper, E. C. (2005). Identification by mass spectrometry and functional characterization of two phosphorylation sites of kcnq2/kcnq3 channels. *Proceedings of the National Academy of Sciences*, 102(49), 17828–17833.
- Sutherland, K. D., Proost, N., Brouns, I., Adriaensen, D., Song, J.-Y., & Berns, A. (2011). Cell of origin of small cell lung cancer: inactivation of trp53 and rb1 in distinct cell types of adult mouse lung. *Cancer cell*, 19(6), 754–764.
- Takeuchi, K., Soda, M., Togashi, Y., Suzuki, R., Sakata, S., Hatano, S., ... others (2012). Ret, ros1 and alk fusions in lung cancer. *Nature medicine*, 18(3), 378–381.

References XL

- Tate John, G., Sally, B., Jubb Harry, C., Zbyslaw, S., Beare David, M., Nidhi, B., ... Elisabeth, D. (2018). Stefancsik ray, thompson sam I, wang shicai, ward sari, campbell peter j, forbes simon a. cosmic: the catalogue of somatic mutations in cancer. *Nucleic Acids Research*, 47(D1), D941–D947.
- Tiffany, H. L., Lautens, L. L., Gao, J.-L., Pease, J., Locati, M., Combadiere, C., ... Murphy, P. M. (1997). Identification of ccr8: a human monocyte and thymus receptor for the cc chemokine i-309. *The Journal of experimental medicine*, 186(1), 165–170.
- Tomasini, P., Barlesi, F., Gilles, S., Nanni-Metellus, I., Soffietti, R., Denicolai, E., ... Metellus, P. (2020). Comparative genomic analysis of primary tumors and paired brain metastases in lung cancer patients by whole exome sequencing: a pilot study. *Oncotarget*, 11(50), 4648.

References XLI

- Travis, W. D. (2002). Pathology of lung cancer. *Clinics in chest medicine*, 23(1), 65–81.
- Tsuji, T., Ficarro, S. B., & Jiang, W. (2006). Essential role of phosphorylation of mcm2 by cdc7/dbf4 in the initiation of dna replication in mammalian cells. *Molecular biology of the cell*, 17(10), 4459–4472.
- Tucker, C. M., Cadotte, M. W., Carvalho, S. B., Davies, T. J., Ferrier, S., Fritz, S. A., ... others (2017). A guide to phylogenetic metrics for conservation, community ecology and macroecology. *Biological Reviews*, 92(2), 698–715.
- Uhrig, S., Ellermann, J., Walther, T., Burkhardt, P., Fröhlich, M., Hutter, B., ... others (2021). Accurate and efficient detection of gene fusions from rna sequencing data. *Genome research*, 31(3), 448–460.

References XLII

- Van der Auwera, G. A., Carneiro, M. O., Hartl, C., Poplin, R., Del Angel, G., Levy-Moonshine, A., ... others (2013). From fastq data to high-confidence variant calls: the genome analysis toolkit best practices pipeline. *Current protocols in bioinformatics*, 43(1), 11–10.
- Vaupel, P., & Mayer, A. (2007). Hypoxia in cancer: significance and impact on clinical outcome. *Cancer and Metastasis Reviews*, 26(2), 225–239.
- Vermi, W., Soncini, M., Melocchi, L., Sozzani, S., & Facchetti, F. (2011). Plasmacytoid dendritic cells and cancer. *Journal of leukocyte biology*, 90(4), 681–690.
- Villarreal, D. O., L'Huillier, A., Armington, S., Mottershead, C., Filippova, E. V., Coder, B. D., ... Princiotta, M. F. (2018). Targeting ccr8 induces protective antitumor immunity and enhances vaccine-induced responses in colon cancer. *Cancer research*, 78(18), 5340–5348.

References XLIII

- Wael, H., Yoshida, R., Kudoh, S., Hasegawa, K., Niimori-Kita, K., & Ito, T. (2014). Notch1 signaling controls cell proliferation, apoptosis and differentiation in lung carcinoma. *Lung Cancer*, 85(2), 131–140.
- Walker, M. A., Pedamallu, C. S., Ojesina, A. I., Bullman, S., Sharpe, T., Whelan, C. W., & Meyerson, M. (2018). Gatk pathseq: a customizable computational tool for the discovery and identification of microbial sequences in libraries from eukaryotic hosts. *Bioinformatics*, 34(24), 4287–4289.
- Wang, B.-Y., Huang, J.-Y., Chen, H.-C., Lin, C.-H., Lin, S.-H., Hung, W.-H., & Cheng, Y.-F. (2020). The comparison between adenocarcinoma and squamous cell carcinoma in lung cancer patients. *Journal of cancer research and clinical oncology*, 146(1), 43–52.

References XLIV

- Wang, C.-Z., Yano, H., Nagashima, K., & Seino, S. (2000). The na⁺-driven cl-/hco₃⁻ exchanger: Cloning, tissue distribution, and functional characterization* 210. *Journal of Biological Chemistry*, 275(45), 35486–35490.
- Wang, L., Darling, J., Zhang, J.-S., Liu, W., Qian, J., Bostwick, D., ... others (2000). Loss of expression of the drr 1 gene at chromosomal segment 3p21. 1 in renal cell carcinoma. *Genes, Chromosomes and Cancer*, 27(1), 1–10.
- Wedepohl, S., Dernedde, J., Vahedi-Faridi, A., Tauber, R., Saenger, W., & Bulut, H. (2017). Reducing macro-and microheterogeneity of n-glycans enables the crystal structure of the lectin and egf-like domains of human l-selectin to be solved at 1.9 Å resolution. *ChemBioChem*, 18(13), 1338–1345.

References XLV

- Wolfson, R. L., Chantranupong, L., Saxton, R. A., Shen, K., Scaria, S. M., Cantor, J. R., & Sabatini, D. M. (2016). Sestrin2 is a leucine sensor for the mtorc1 pathway. *Science*, 351(6268), 43–48.
- Wu, Z., Fournel, L., Stadler, N., Liu, J., Boullier, A., Hoyeau, N., ... others (2019). Modulation of lung cancer cell plasticity and heterogeneity with the restoration of cisplatin sensitivity by neurotensin antibody. *Cancer letters*, 444, 147–161.
- Wyles, J. P., Wu, Z., Mirski, S. E., & Cole, S. P. (2007). Nuclear interactions of topoisomerase ii α and β with phospholipid scramblase 1. *Nucleic acids research*, 35(12), 4076–4085.
- Xia, H.-W., Zhang, Z.-Q., Yuan, J., & Niu, Q.-L. (2021). Human recql5 promotes metastasis and resistance to cisplatin in non-small cell lung cancer. *Life Sciences*, 265, 118768.

References XLVI

- Xiao, D., Li, F., Pan, H., Liang, H., Wu, K., & He, J. (2017). Integrative analysis of genomic sequencing data reveals higher prevalence of *lrp1b* mutations in lung adenocarcinoma patients with copd. *Scientific reports*, 7(1), 1–8.
- Xiao, H., He, M., Xie, G., Liu, Y., Zhao, Y., Ye, X., ... Zhang, M. (2019). The release of tryptase from mast cells promote tumor cell metastasis via exosomes. *BMC cancer*, 19(1), 1–9.
- Xie, M., Zhang, L., He, C.-s., Xu, F., Liu, J.-l., Hu, Z.-h., ... Tian, Y. (2012). Activation of notch-1 enhances epithelial–mesenchymal transition in gefitinib-acquired resistant lung cancer cells. *Journal of cellular biochemistry*, 113(5), 1501–1513.
- Yalla, K., Elliott, C., Day, J. P., Findlay, J., Barratt, S., Hughes, Z. A., ... others (2018). *Fbxw7* regulates *disc1* stability via the ubiquitin-proteosome system. *Molecular psychiatry*, 23(5), 1278–1286.

References XLVII

- Ye, T., Yang, X., Liu, H., Lv, P., & Ye, Z. (2020). Long non-coding rna blacat1 in human cancers. *OncoTargets and therapy*, 13, 8263.
- Yeh, C.-H., Bellon, M., & Nicot, C. (2018). Fbxw7: a critical tumor suppressor of human cancers. *Molecular cancer*, 17(1), 1–19.
- Yin, J., Kwon, Y. T., Varshavsky, A., & Wang, W. (2004). Recql4, mutated in the rothmund–thomson and rapadilino syndromes, interacts with ubiquitin ligases ubr1 and ubr2 of the n-end rule pathway. *Human molecular genetics*, 13(20), 2421–2430.
- Yokobori, T., Yokoyama, Y., Mogi, A., Endoh, H., Altan, B., Kosaka, T., ... others (2014). Fbxw7 mediates chemotherapeutic sensitivity and prognosis in nsclcs. *Molecular Cancer Research*, 12(1), 32–37.
- Zeng, S., Liu, A., Dai, L., Yu, X., Zhang, Z., Xiong, Q., ... others (2019). Prognostic value of top2a in bladder urothelial carcinoma and potential molecular mechanisms. *BMC cancer*, 19(1), 1–12.

References XLVIII

- Zhang, J., Shao, J., Zhu, L., Zhao, R., Xing, J., Wang, J., ... Yu, K. (2017). Molecular profiling identifies prognostic markers of stage ia lung adenocarcinoma. *Oncotarget*, 8(43), 74846.
- Zhou, H., Zhang, C., Li, H., Chen, L., & Cheng, X. (2020). A novel risk score system of immune genes associated with prognosis in endometrial cancer. *Cancer cell international*, 20(1), 1–12.
- Zhu, T.-T., Sun, R.-L., Yin, Y.-L., Quan, J.-P., Song, P., Xu, J., ... Li, P. (2019). Long noncoding rna uca1 promotes the proliferation of hypoxic human pulmonary artery smooth muscle cells. *Pflügers Archiv-European Journal of Physiology*, 471(2), 347–355.
- Zhu, X., Chen, H., Yang, Y., Xu, C., Zhou, J., Zhou, J., & Chen, Y. (2018). Distinct prognosis of mrna expression of the five recql dna-helicase family members—recql, blm, wrn, recql4, and recql5—in patients with breast cancer. *Cancer management and research*, 10, 6649.