Genomic characterization of early-stage esophageal squamous cell carcinoma in a Japanese population

SUPPLEMENTARY MATERIALS

Supplementary Table 1: Sample characteristics of 42 superficial esophageal squamous cell neoplasm patients

ESCN[a](HGIN/T1a/T1b)	6/24/12
Gender (Male/Female)	39/3
Age	68.4 ± 7.0 (53–83)
Drinking History (none/light/heavy)[b]	1/16/25
smoking (none/light/heavy)[c]	3/21/18
rs671 at ALDH2 (GA/GG+AA)	29/13
rs1229984 at ADH1B (GG/GA+AA)	5/37

[b]Heavy alcohol drinker was defined as alcohol consumption of more than 60 g/day based on the ethanol conversion data.
[c]Heavy smoker was defined as tobacco consumption more than the Brickman index 1000.
[a]ESCN; Esophageal squamous cell neoplasm.

Supplementary Table 2: Average read depth for exome sequencing in 16 ESCC samples

	Average Coverage	
ID	Tumor	Blood
26723	186.6	105.4
11515	139.6	101.7
22780	127.5	107
23727	74.2	75.4
25178	142	128.8
25287	118.5	131.8
11489	166.4	122.4
22739	159.3	68.8
23658	132	110.2
24311	140.4	87.5
24411	150.1	84.7
25074	144.7	85.2
25243	141.4	58.9
22833	120.7	91.8
26694	187.6	123.4
25089	164.1	143

Average read Depth for exome sequencing in 16 esophageal squamous cell carcinoma and matched blood leukemia samples. Results were analyzed using exome sequencing.

Supplementary Table 3: The frequencies covered by more than 10 sequences in screening stage samples

	The frequency of target bases covered were > 10 (%)	
ID	Tumor	Blood
26723	98.39	99.1
11515	98.88	98.51
22780	98.78	98.52
23727	84.74	97.61
25178	98.84	98.74
25287	98.65	98.78
11489	98.99	98.77
22739	98.95	97.03
23658	98.7	98.58
24311	98.87	98.03
24411	98.85	97.92
25074	98.78	98.16
25243	98.82	81.75
22833	98.61	98.23
26694	99.04	98.52
25089	98.71	98.99

Target bases were covered by more than 10 sequences during exome sequencing of 16 esophageal squamous cell carcinoma and matched blood leukemia samples. Results were analyzed by exome sequencing.

Supplementary Table 4: Somatic mutations in 16 early-stage Esophageal neoplasms using exome sequecncing. See Supplementary_Table_4

Supplementary Table 5: Taget panel mounted 128 genes used in the replication stage

AJUBA	*FAT2*	*KRAS*	*NOTHC3*	*SLC6A6*	*ZNF323*
BAP1	*FAT3*	*LAMB1*	*NUTM2G*	*SLITRK3*	*ZNF496*
BMPR1B	*FAT4*	*LIMCH1*	*OBSCN*	*SOX1*	*ZNF750*
C17orf28	*FBLN2*	*LRP1B*	*OR2T3*	*SOX2*	
CABS1	*FBRS*	*LRP5*	*PALB2*	*STAG2*	
CCND1	*FLG*	*LRPAP1*	*PCDH8*	*SYBU*	
CDH23	*FOXA1*	*LYST*	*PCDHA1*	*TDRD1*	
CDKN2B	*FOXC2*	*MAGEE1*	*PCDHA13*	*TERT*	
CES1	*FRYL*	*MET*	*PCDHA3*	*TET1*	
COL6A6	*FSCB*	*MGA*	*PCLO*	*TET2*	
CREBBP	*GPR98*	*MIA3*	*PIK3CA*	*TGFBR2*	
CSMD1	*GTPBP8*	*MLL1*	*PKHD1L1*	*THSD7B*	
CSMD3	*HBG2*	*MLL2*	*PRDM9*	*TLR1*	
CUL9	*HERC6*	*MSTO1*	*PRSS21*	*TP53*	
DCSTAMP	*HKR1*	*MTERFD1*	*PTEN*	*TTN*	
DHX9	*HPSE*	*MUC16*	*PTPN13*	*UBR2*	
DNAH14	*HUWE1*	*MUC19*	*PTPRD*	*ULK4*	
DNAH3	*HYDIN*	*MYO18A*	*RAG1*	*UNC13C*	
DST	*INTU*	*NBEA*	*RB*	*VPS35*	
EGFR	*IQCB1*	*NCKAP5*	*RELN*	*WAPAL*	
EP300	*IQSEC1*	*NCOR1*	*RFX6*	*WNK2*	
ERBB2	*ITGA10*	*NFE2L2*	*SACS*	*YAP1*	
F5	*KDM6A*	*NLRP14*	*SDAD1*	*ZAN*	
FAM135B	*KIR3DL1*	*NOTCH1*	*SIDT1*	*ZCCHC2*	
FAT1	*KLF5*	*NOTCH2*	*SLC35E1*	*ZFHX4*	

Target panel-mounted 128 genes used in the replication stage. These 128 genes included 89 genes that had non-synonymous somatic mutations and copy-number alterations in at least two patients, and 39 genes were associated with the development of Japanese esophageal squamous cell carcinoma.

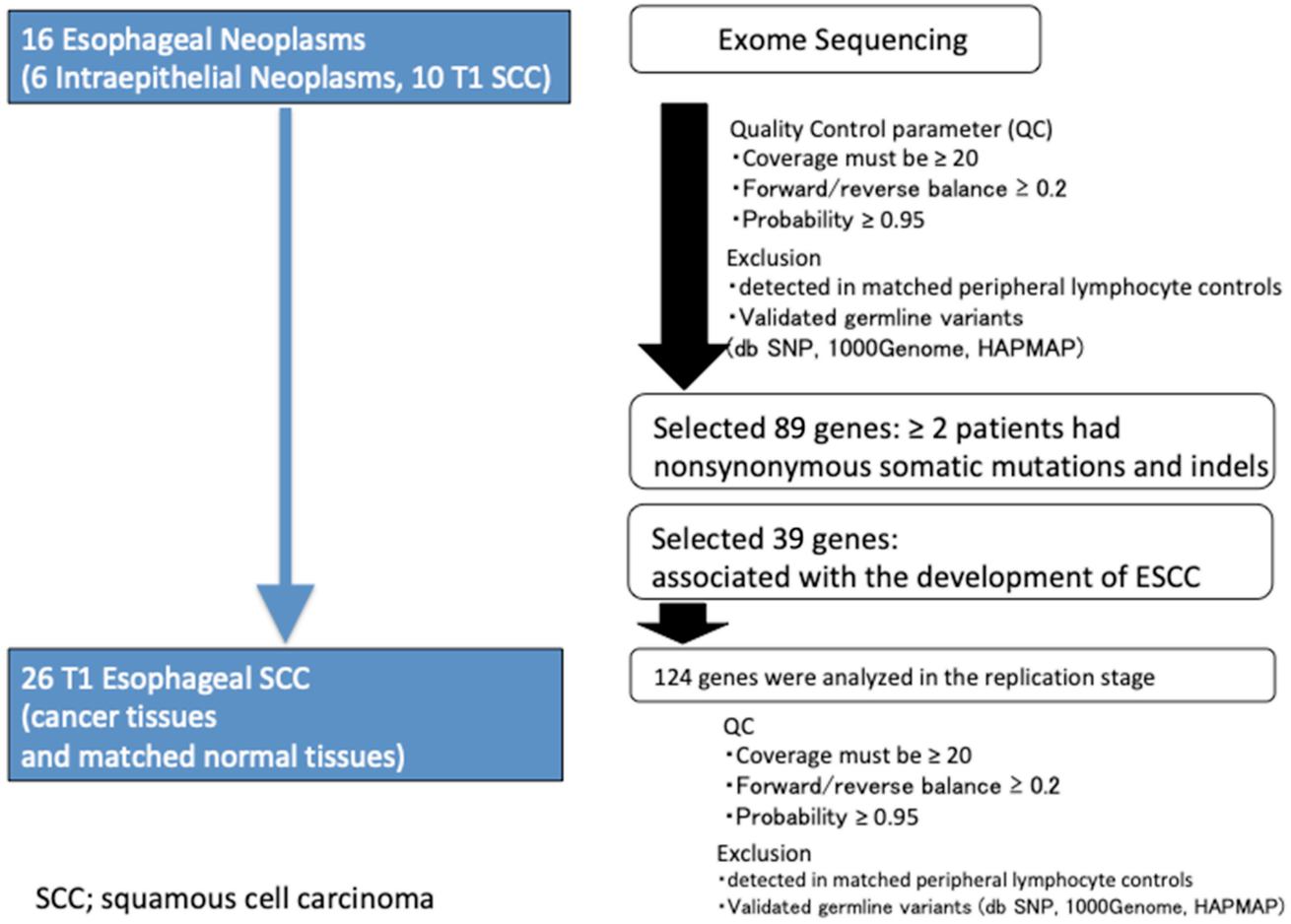
Supplementary Table 6: Somatic mutations in 26 cancerous tissues in early-stage Esophageal squamous cell carcinoma using target sequecncing. See Supplementary_Table_6

Supplementary Table 7: Somatic mutations in 26 non-cancerous tissues in early-stage esophageal squamous cell carcinoma using target sequencing

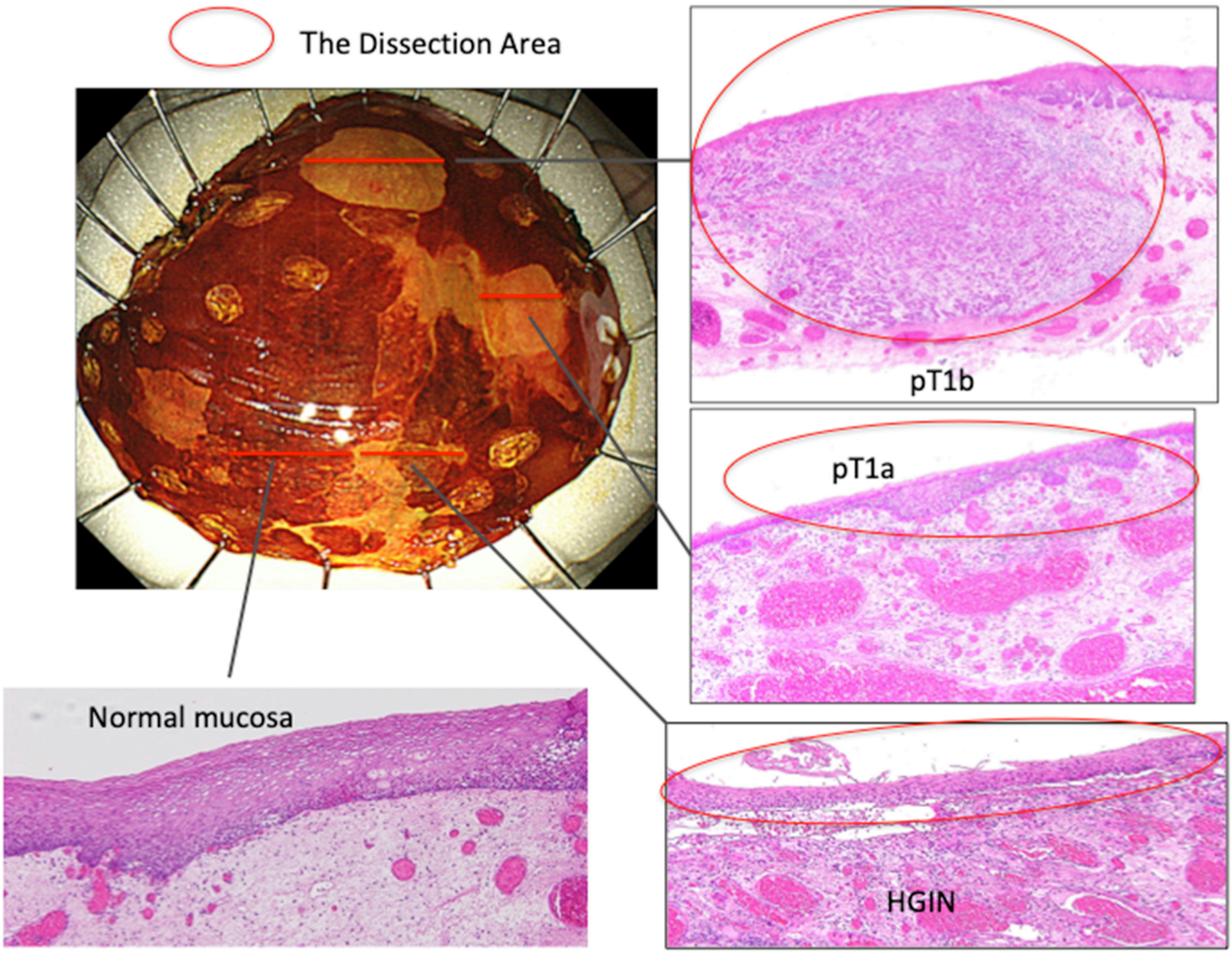
Sample ID	Gene	Type	Zygosity	Chr.	Position	Ref	Alt	MAF	Count	Coverage	Codon	AA	AA
8372	NOTCH2	SNP	HET	1	120478094	C	A	0.308	20	65	MISSENSE	gGt/gTt	G1219V
8372	TTN	SNP	HOM	2	179449478	G	T	0.205	9	44	NONSENSE	taC/taA	Y12565*
8372	FAM135B	SNP	HOM	8	139164848	G	C	0.131	49	374	MISSENSE	Caa/Gaa	Q624E
8372	NOTCH1	SNP	HOM	9	139399229	C	T	0.219	33	151	NONSENSE	tgG/tgA	W1638*
8372	TP53	SNP	HET	17	7578478	G	C	0.511	95	186	MISSENSE	cCc/cGc	P112R
8399	ULK4	SNP	HOM	3	41996187	C	A	0.133	4	30	MISSENSE	cGa/cTa	R22L
8399	EGFR	SNP	HOM	7	55233129	G	T	0.105	4	38	NONSENSE	Gga/Tga	G627*
8420	CUL9	SNP	HOM	6	43170869	G	T	0.121	4	33	MISSENSE	aGc/aTc	S1259I
8420	EGFR	SNP	HOM	7	55260487	C	A	0.111	4	36	NONSENSE	tCa/tAa	S885*
8420	PCLO	SNP	HOM	7	82545627	G	T	0.125	4	32	MISSENSE	tCc/tAc	S3892Y
8496	MUC19	SNP	HOM	12	40879080	C	T	0.216	66	306	MISSENSE	gCa/gTa	A4109V
8496	MUC19	SNP	HOM	12	40879082	A	T	0.216	66	306	NONSENSE	Aga/Tga	R4110*
26710	MIA3	SNP	HOM	1	222838725	C	T	0.185	22	119	MISSENSE	Ctc/Ttc	L1830F
26710	MIA3	SNP	HOM	1	222838734	C	T	0.212	21	99	MISSENSE	Cgg/Tgg	R1833W
26710	TTN	SNP	HOM	2	179632555	G	T	0.121	4	33	MISSENSE	aaC/aaA	N3088K
26710	ZAN	SNP	HOM	7	100374121	C	T	0.207	12	58	MISSENSE	gCg/gTg	A2139V
26710	CLUH	SNP	HET	17	2594021	A	T	0.264	42	159	MISSENSE	cTc/cAc	L1266H
26710	TP53	SNP	HOM	17	7577586	A	C	0.198	118	595	MISSENSE	aTc/aGc	I100S
27487	AKR7L	SNP	HOM	1	19600376	T	G	0.119	5	42	MISSENSE	Atg/Ctg	M65L
27487	FLG	SNP	HOM	1	152280857	T	C	0.107	6	56	MISSENSE	Agc/Ggc	S2169G
27487	MUC19	SNP	HOM	12	40879080	C	T	0.201	40	199	MISSENSE	gCa/gTa	A4109V
27487	MUC19	SNP	HOM	12	40879082	A	T	0.201	40	199	NONSENSE	Aga/Tga	R4110*
27487	NBEA	Deletion	HET	13	35516972	GCCG GGC	G	0.662	45	68		ccgggc/-	PG6-
27512	PCDH17	Deletion	HOM	13	58299434	GA	G	0.169	11	65			
27554	PCDHB6	SNP	HOM	5	140532121	G	T	0.118	4	34	MISSENSE	gaG/gaT	E625D
27554	TP53	Insertion	HOM	17	7578394	T	TGGTG GGGGCA	0.138	34	247			
27567	F5	SNP	HOM	1	169515742	C	A	0.133	4	30	MISSENSE	tGt/tTt	C567F
27567	IQCB1	SNP	HOM	3	121500705	G	A	0.125	4	32	MISSENSE	gCg/gTg	A299V
27567	MUC19	SNP	HET	12	40879854	G	T	0.467	28	60	MISSENSE	aGc/aTc	S4367I
27581	F5	SNP	HOM	1	169529927	C	A	0.105	4	38	NONSENSE	Gaa/Taa	E151*
27581	PCDH18	SNP	HOM	4	138451277	C	A	0.108	4	37	MISSENSE	Gtt/Ttt	V656F
27581	MUC19	SNP	HOM	12	40879854	G	T	1	34	34	MISSENSE	aGc/aTc	S4367I
27584	TTN	SNP	HET	2	179430997	G	A	0.357	5	14	MISSENSE	aCg/aTg	T17556M
27584	FAM135B	SNP	HOM	8	139149456	G	T	0.105	4	38	MISSENSE	Cgt/Agt	R1317S
27596	CSPG4	SNP	HOM	15	75982072	G	A	0.103	6	58	MISSENSE	gCc/gTc	A445V
27596	CSPG4	SNP	HOM	15	75982085	C	T	0.105	6	57	MISSENSE	Gag/Aag	E441K
27599	TP53	SNP	HOM	17	7577081	T	C	0.138	30	218	MISSENSE	gAa/gGa	E127G
28463	TTN	SNP	HOM	2	179463986	G	A	0.129	4	31	MISSENSE	aCg/aTg	T16277M
28463	MUC19	SNP	HOM	12	40879080	C	T	0.153	24	157	MISSENSE	gCa/gTa	A4109V
28463	MUC19	SNP	HOM	12	40879082	A	T	0.152	24	158	NONSENSE	Aga/Tga	R4110*
28524	TTN	SNP	HOM	2	179474027	C	A	0.105	4	38	MISSENSE	cGa/cTa	R14769L
28524	CSPG4	SNP	HOM	15	75982085	C	T	0.196	10	51	MISSENSE	Gag/Aag	E441K
28643	MUC19	SNP	HOM	12	40879080	C	T	0.212	50	236	MISSENSE	gCa/gTa	A4109V
28643	MUC19	SNP	HOM	12	40879082	A	T	0.211	50	237	NONSENSE	Aga/Tga	R4110*
28643	HUWE1	SNP	HOM	X	53610712	G	A	0.121	4	33	MISSENSE	Cat/Tat	H1776Y
28773	PRG4	SNP	HOM	1	186277277	G	A	0.144	115	801	MISSENSE	gGg/gAg	G675E
28773	PCDHB8	SNP	HET	5	140558212	A	C	0.273	12	44	MISSENSE	aaA/aaC	K199N
28773	ZNF750	SNP	HOM	17	80790256	A	T	0.113	59	520	NONSENSE	taT/taA	Y25*
28909	PCDHB2	SNP	HOM	5	140474669	G	A	0.118	4	34	MISSENSE	Ggc/Agc	G99S

29019	ULK4	SNP	HOM	3	41942307	G	T	0.108	4	37	MISSENSE	caC/caA	H399Q
29019	FAT1	SNP	HET	4	187532585	T	C	0.25	11	44	MISSENSE	Ata/Gta	I3270V
29019	ADGRV1	SNP	HOM	5	90449044	C	A	0.105	4	38	MISSENSE	Cct/Act	P6211T
29019	CSMD3	SNP	HOM	8	113314108	C	A	0.111	4	36	MISSENSE	tGc/tTc	C2616F
29019	SACS	SNP	HOM	13	23912502	C	A	0.125	4	32	MISSENSE	gGa/gTa	G1691V
29019	TP53	SNP	HET	17	7578235	T	C	0.399	144	361	MISSENSE	tAt/tGt	Y166C
29019	MUC16	SNP	HOM	19	9059971	C	T	0.174	33	190	MISSENSE	Gtt/Att	V9159I
29019_IN	DHX9	SNP	HET	1	182850535	G	T	0.447	59	132	MISSENSE	Gta/Tta	V1025L
29019_IN	CAMSAP2	SNP	HOM	1	200776609	C	G	0.179	7	39	MISSENSE	Caa/Gaa	Q169E
29019_IN	LRP1B	SNP	HOM	2	141660668	A	G	0.183	17	93	MISSENSE	gTc/gCc	V1196A
29019_IN	TTN	SNP	HET	2	179659753	C	T	0.357	96	269	MISSENSE	Ggt/Agt	G381S
29019_IN	BAP1	SNP	HET	3	52443861	G	C	0.618	55	89	MISSENSE	Cca/Gca	P12A
29019_IN	PKHD1L1	SNP	HET	8	110410707	G	C	0.253	56	221	MISSENSE	ttG/ttC	L314F
29019_IN	NOTCH1	SNP	HET	9	139399276	C	A	0.572	115	201	NONSENSE	Gag/Tag	E1623*
29019_IN	TP53	SNP	HOM	17	7578272	G	A	0.762	80	105	MISSENSE	Cat/Tat	H154Y
29829	MUC19	SNP	HOM	12	40879080	C	T	0.125	32	256	MISSENSE	gCa/gTa	A4109V
29829	MUC19	SNP	HOM	12	40879082	A	T	0.125	32	256	NONSENSE	Aga/Tga	R4110*
29829	MUC19	SNP	HET	12	40879854	G	T	0.531	34	64	MISSENSE	aGc/aTc	S4367I
29845	TTN	SNP	HET	2	179441732	G	T	0.283	15	53	NONSENSE	taC/taA	Y14045*
29845	FAT1	SNP	HOM	4	187549851	C	G	0.235	8	34	MISSENSE	Gtt/Ctt	V1464L
29845	NOTCH1	SNP	HET	9	139395021	C	G	0.45	54	120	MISSENSE	Gca/Cca	A1973P
29850	FLG-AS1	SNP	HOM	1	152286367	C	A	0.167	6	36	MISSENSE	Cca/Aca	P145T
29850	PRG4	SNP	HOM	1	186277277	G	A	0.143	101	708	MISSENSE	gGg/gAg	G675E
29850	PCDHA8	SNP	HOM	5	140221012	C	T	0.2	17	85	MISSENSE	Ccc/Tcc	P36S
29850	CUL9	SNP	HOM	6	43184132	A	C	0.108	8	74	MISSENSE	cAc/cCc	H2058P
29850	MUC16	SNP	HOM	19	9018508	T	G	0.127	8	63	MISSENSE	Aag/Cag	K12556Q
29855	PCDHA8	SNP	HOM	5	140221071	G	T	0.231	15	65	MISSENSE	gaG/gaT	E55D
29855	APOBEC3B	SNP	HOM	22	39381835	T	C	0.138	18	130	MISSENSE	Tac/Cac	Y65H

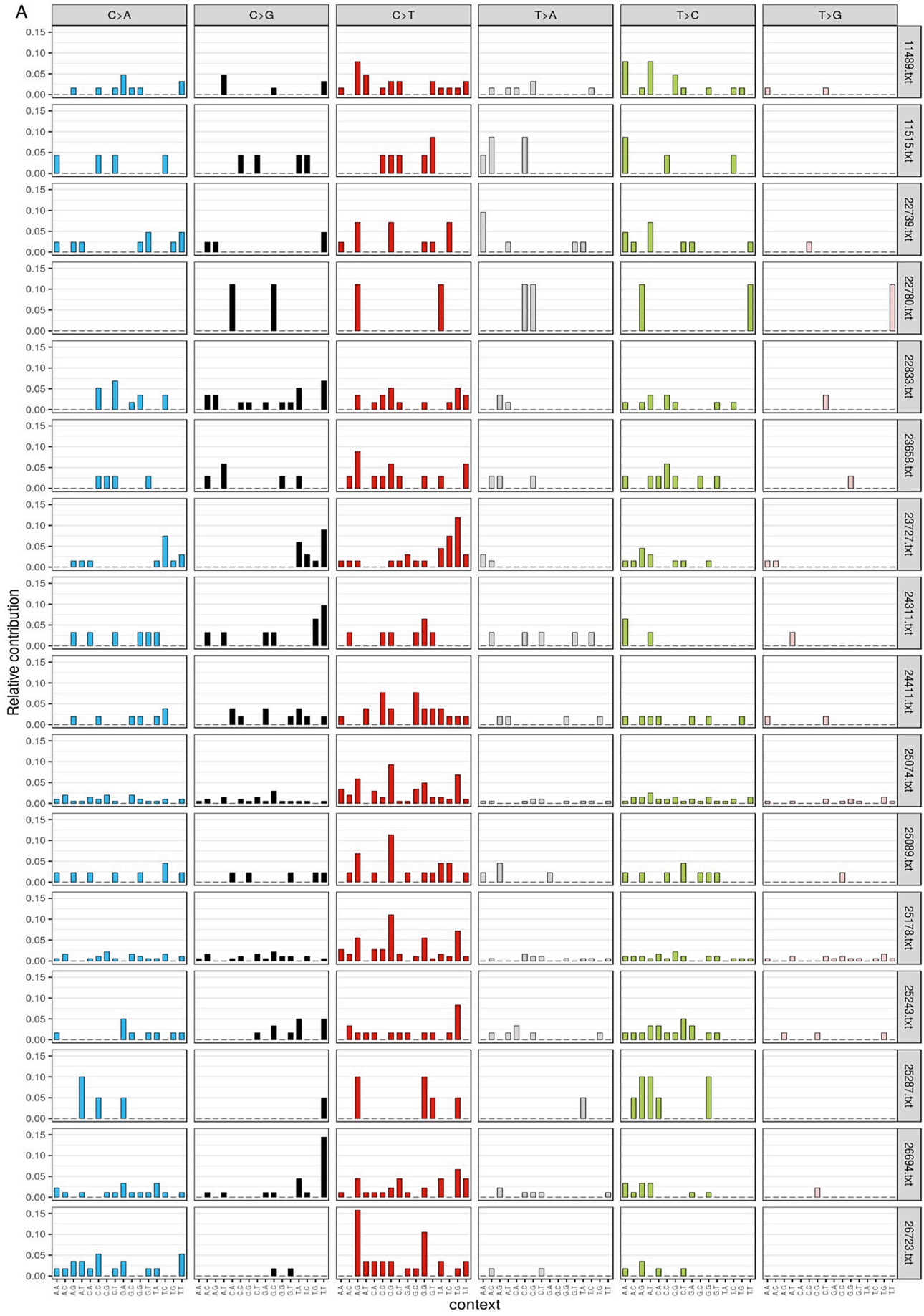
Non-synonymous somatic mutations in 26 normal mucosae from early-stage esophageal squamous cell carcinoma (ESCC).
Patients identified using target panel mounted with candidate ESCC driver genes.
Chr, Chromosome; Ref, Reference allele; Alt, mutant allele; MAF, mutant allele freguency; Coverage is filter read depth using molecular barcode.



Supplementary Figure 1: Study design.



Supplementary Figure 2: Diagram showing the hematoxylin- and eosin-stained sections of morphologically normal epithelial tissue, low-grade dysplasia (LD), high-grade dysplasia (HD), and invasive esophageal squamous cell carcinoma (ESCC) from the same case.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Relative contribution
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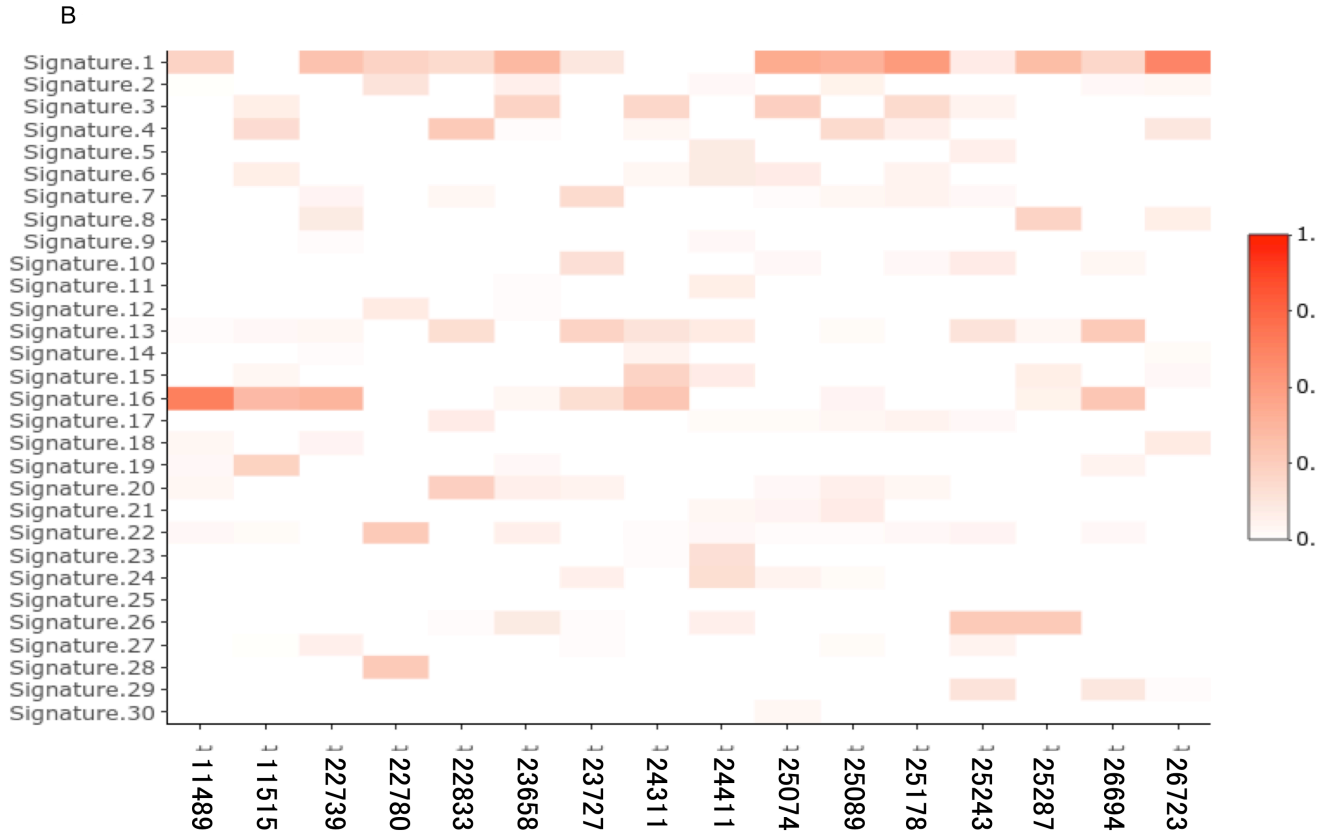
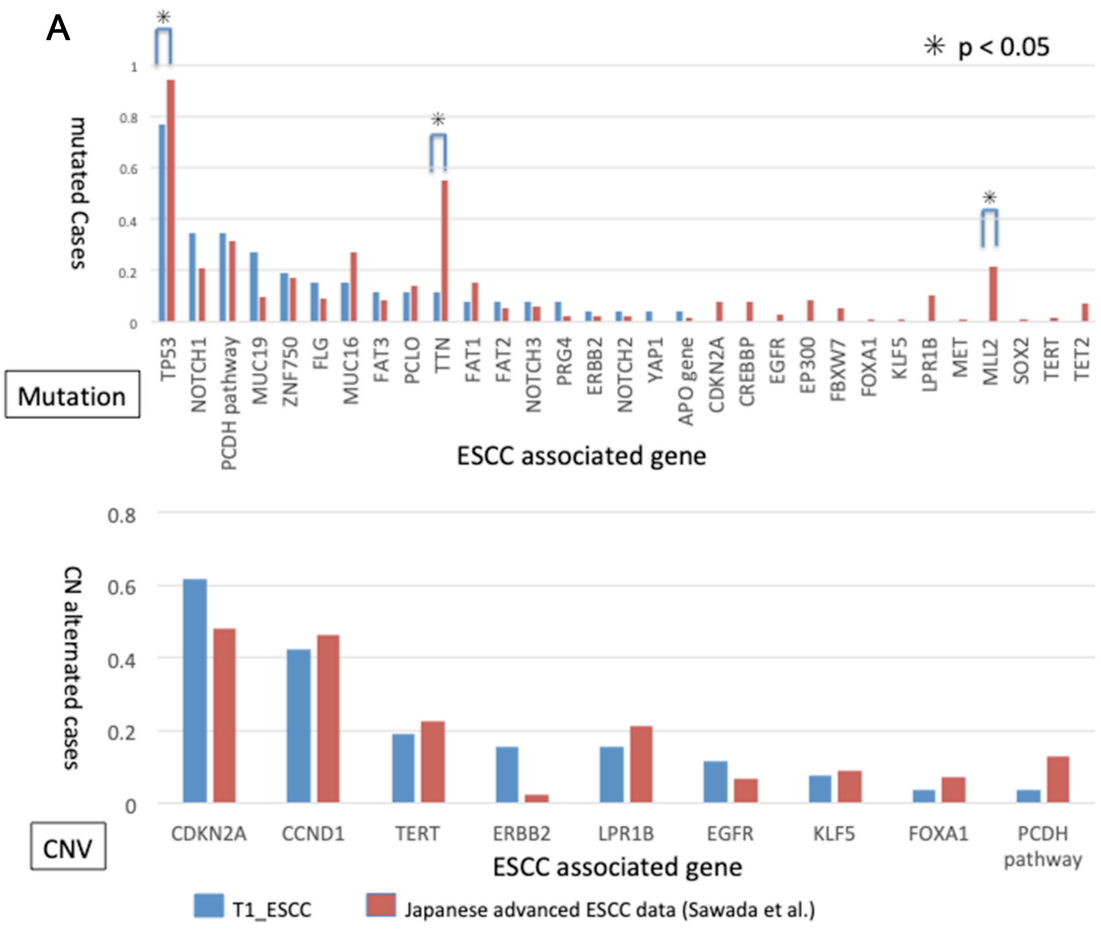
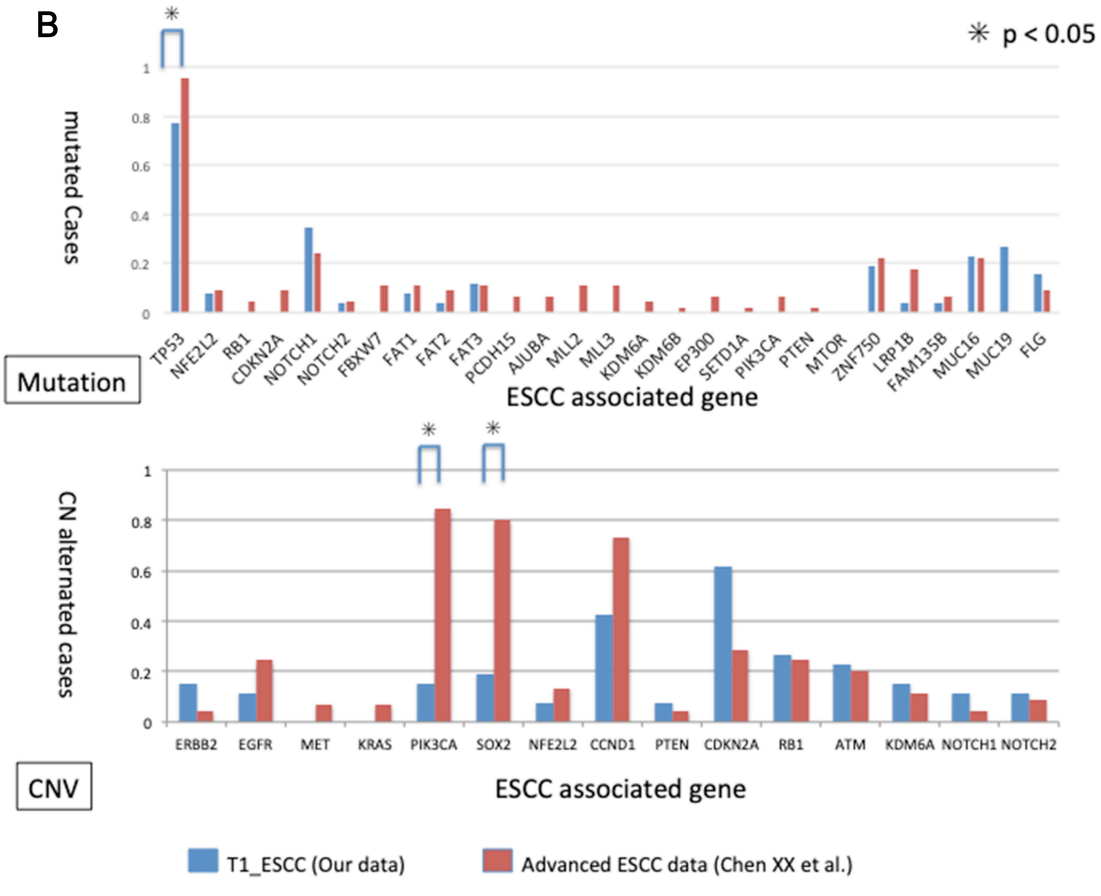
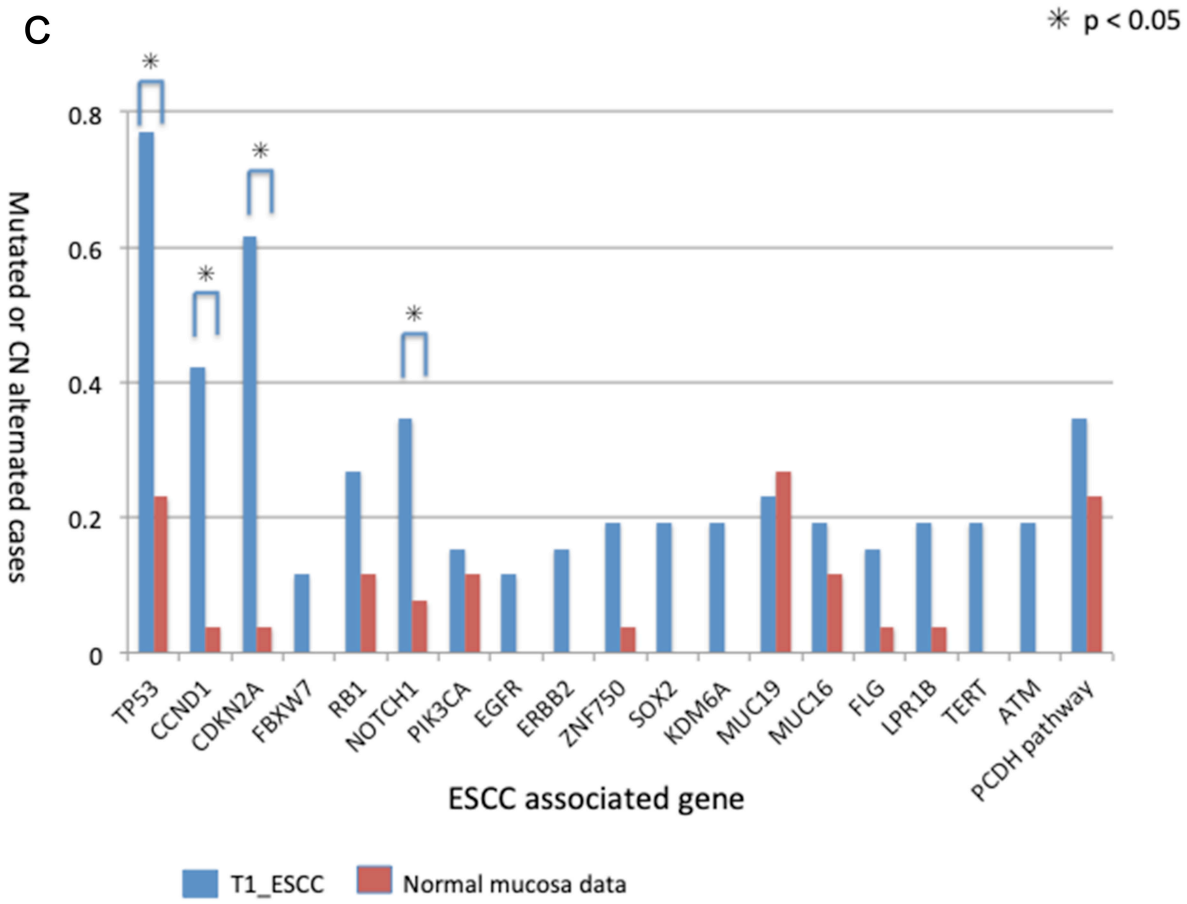
0.05
0.00
context



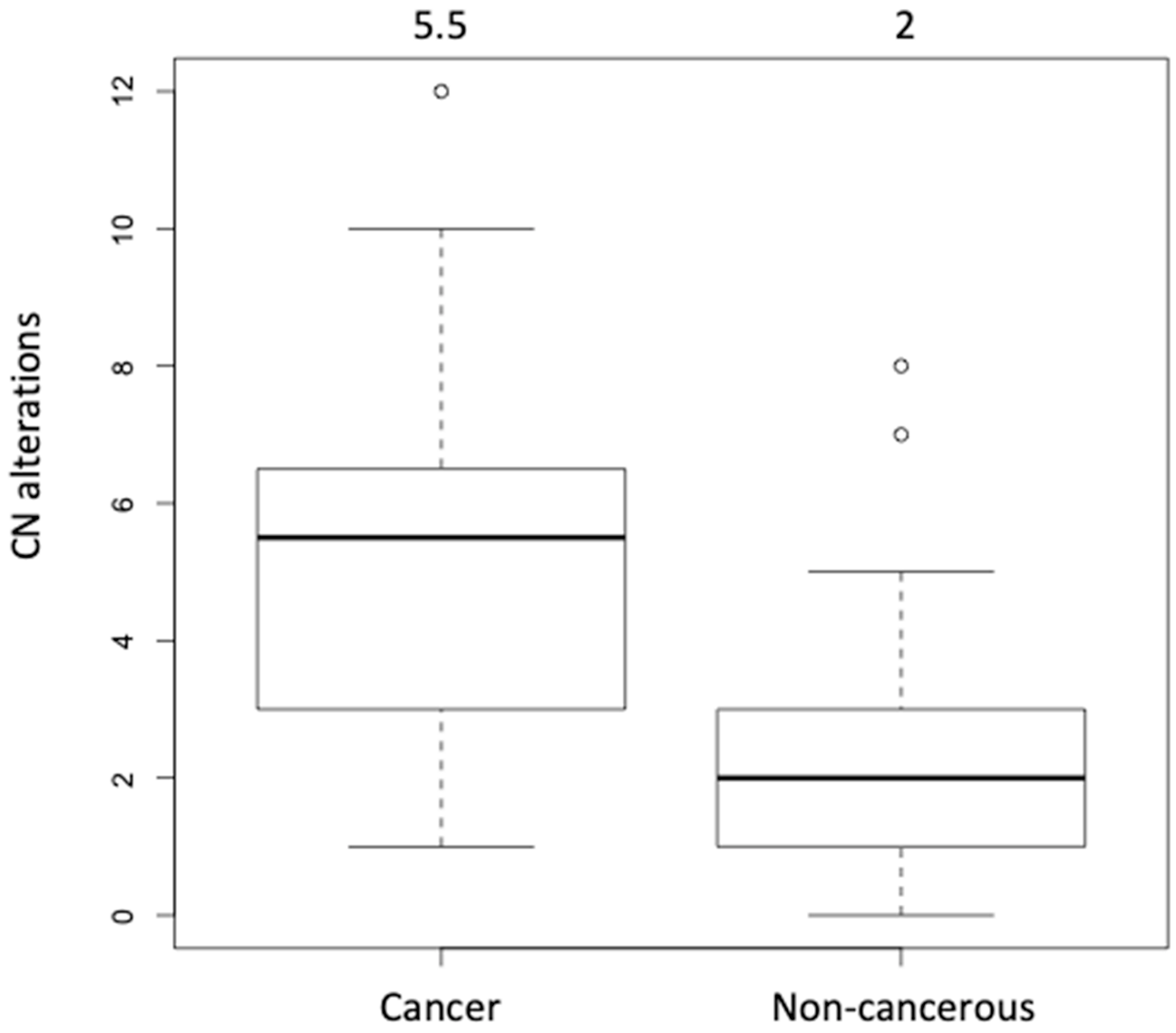
Supplementary Figure 3: Mutation signatures of ESCC samples used during the screening stage. (**A**) Number of trinucleotide substitution patterns in esophageal squamous cell neoplasms. Six possible substitutions from pyrimidine bases were further subdivided into 96 patterns based on the neighboring nucleotides. (**B**) PCA plot of mutational signatures.


A
* p < 0.05
mutated Cases
Mutation
TP53
NOTCH1
PCDH pathway
MUC19
ZNF750
FLG
MUC16
FAT3
PCLO
TTN
FAT1
FAT2
NOTCH3
PRG4
ERBB2
NOTCH2
YAP1
APO gene
CDKN2A
CREBBP
EGFR
EP300
FBXW7
FOXA1
KLF5
LPR1B
MET
MLL2
SOX2
TERT
TET2
ESCC associated gene
CN alternated cases
CNV
CDKN2A
CCND1
TERT
ERBB2
LPR1B
EGFR
KLF5
FOXA1
PCDH pathway
ESCC associated gene
T1_ESCC
Japanese advanced ESCC data (Sawada et al.)


B
* p < 0.05
mutated Cases
Mutation
TP53
NFE2L2
RB1
CDKN2A
NOTCH1
NOTCH2
FBXW7
FAT1
FAT2
FAT3
PCDH15
AJUBA
MLL2
MLL3
KDM6A
KDM6B
EP300
SETD1A
PIK3CA
PTEN
MTOR
ZNF750
LRP1B
FAM135B
MUC16
MUC19
FLG
ESCC associated gene
CN alternated cases
CNV
ERBB2
EGFR
MET
KRAS
PIK3CA
SOX2
NFE2L2
CCND1
PTEN
CDKN2A
RB1
ATM
KDM6A
NOTCH1
NOTCH2
ESCC associated gene
T1_ESCC (Our data)
Advanced ESCC data (Chen XX et al.)



Supplementary Figure 4: (**A**) Comparative analysis of the frequency of non-synonymous somatic mutations and copy-number alterations in early stage and advanced-stage cancer [7] in representative esophageal squamous cell carcinoma (ESCC) driver genes. (**B**) Comparative analysis of the frequency of non-synonymous somatic mutations and copy-number alterations in early stage ESCC and advanced-stage cancer [16] in representative esophageal squamous cell carcinoma (ESCC) driver genes. (**C**) Comparative analysis of the frequency of non-synonymous somatic mutations between early-stage esophageal squamous cell carcinoma (ESCC) and non-cancerous tissue in each representative gene. The early stage ESCC and non-cancerous data from 26 ESCC patients with mutations in each representative ESCC driver gene were collected only during the replication stage.



Supplementary Figure 5: Comparative analysis of copy-number alterations in early-stage esophageal squamous cell carcinoma tissues and matched non-cancerous tissues in 26 replication-stage patients.