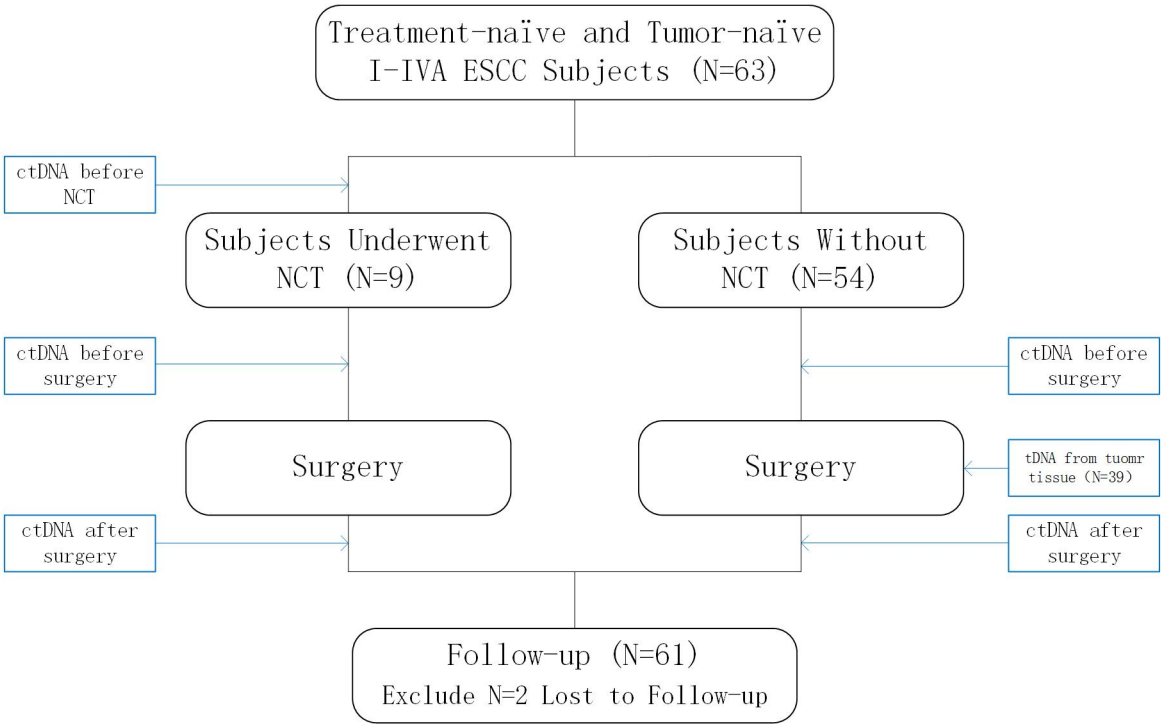
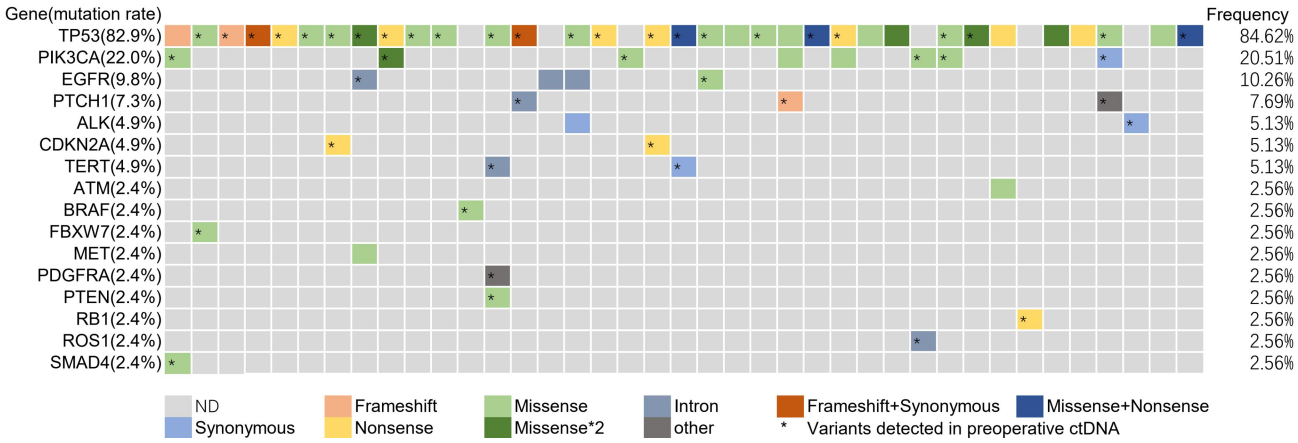
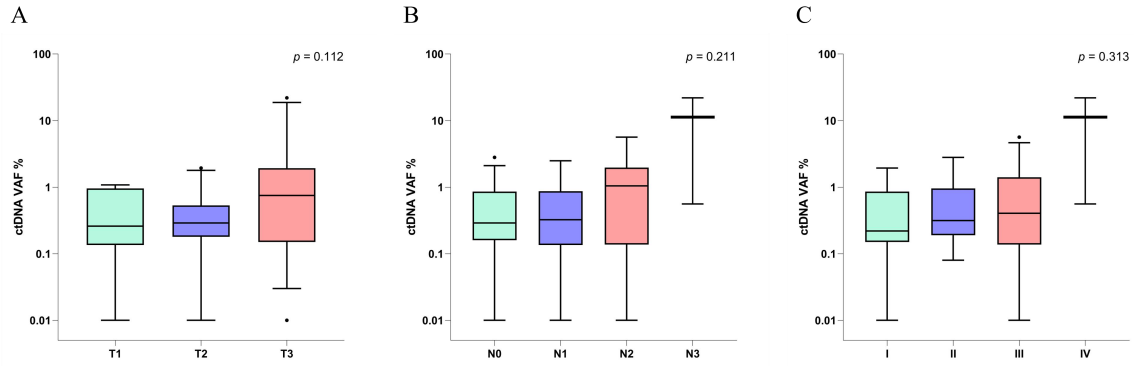


Supplementary Figure 1 Overview of patient enrollment and sample collection. ESCC: Esophageal squamous cell carcinoma; NCT: Neo adjuvant chemotherapy.



Supplementary Figure 2 Somatic mutations in ESCC tumor tissue from 39 samples. ESCC: Esophageal squamous cell carcinoma.



Supplementary Figure 3 Association between preoperative ctDNA VAF (tumor-agnostic) and pathological staging in ESCC patients. ESCC: Esophageal squamous cell carcinoma; VAF: Mutation abundance; ctDNA not detected: Shown as 0.01% on log scale.

Supplementary Table 1 The panel of 61 genes

The panel of 61 genes						
AR	*CDK6*	*EZH2*	*GNAS*	*KRAS*	*PDGFRA*	*SMAD4*
ABL1	*CDKN2A*	*FBXW7*	*HNF1A*	*MAP2K1*	*PIK3CA*	*SMACRB1*
AKT1	*CSF1R*	*FGFR1*	*HRAS*	*MET*	*PTCH1*	*SMO*
ALK	*CTNNB1*	*FGFR2*	*IDH1*	*MLH1*	*PTEN*	*SRC*
APC	*DDR2*	*FGFR3*	*IDH2*	*MPL*	*PTPN11*	*TERT*
ATM	*DNMT3A*	*FLT3*	*JAK2*	*MSH6*	*RB1*	*TP53*
BRAF	*EGFR*	*FOXL2*	*JAK3*	*NOTCH1*	*RET*	*TSC1*
CDH1	*ERBB2*	*GNA11*	*KDR*	*NPM1*	*ROS1*	
CDK4	*ERBB4*	*GNAQ*	*KIT*	*NRAS*	*STK11*	

Supplementary Table 2 Patient characteristics

Variable	Case (n = 63)	Percentage (%)
Age		
Median (IQR)	65.0 (60.5 - 70.0)	
Gender		
Male	51	80.95
Female	12	19.05
BMI		
Median (IQR)	23.7 (22.1 - 24.9)	
Tobacco and alcohol use		
Both	26	41.27
Tobacco only	11	17.46
Alcohol only	3	4.76
None	23	36.51
Tumor location		
Upper	6	9.52
Middle	40	63.49
Lower	17	26.98
Surgery procedure		
Sweet	4	6.35
Ivor lewis	49	77.78
Mckeown	10	15.87
Maximum tumor diameter		
Median (IQR)	3.5 (2.4 - 4.5)	
Tumor differentiation		
G1	8	12.70
G2	44	69.84
G3	11	17.46
Tumor stage		

T1	12	19.05
T2	28	44.44
T3	23	36.51
Node stage		
N0	35	55.56
N1	16	25.40
N2	10	15.87
N3	2	3.17
pTNM stage		
I	19	30.16
II	16	25.40
III	26	41.27
IV	2	3.17
Angiolymphatic invasion		
Yes	14	22.22
No	49	77.78
Neurological invasion		
Yes	13	20.63
No	50	79.37
Neoadjuvant therapy		
Chemotherapy	9	14.29
No	54	85.71
Adjuvant therapy		
Chemotherapy	12	19.05
Chemoradiotherapy	7	11.11
Radiotherapy	7	11.11
No	37	58.73

Supplementary Table 3. Discordance between preoperative ctDNA and matched tumor DNA in 8 ESCC cases.

Patient ID	Gene	Mutation	Type	tDNA VAF	Pre-surg ctDNA VAF	Post-surg ctDNA VAF
CHE022	ATM	ATM:p.T2438I	Missense	-	0.15%	0.13%
	ERBB2	ERBB2:p.A657V	Missense	-	0.08%	-
	HNF1A	HNF1A:p.R229Q	Missense	-	0.19%	-
	PTEN	PTEN:p.P339A	Missense	-	0.22%	-
	EGFR	chr7:g.55269062G>C	Intron	9.56%	-	-
CHE031	AR	AR:p.P379L	Missense	-	0.57%	-
	TP53	TP53:p.R248Q	Missense	28.18%	-	-
CHE039	ATM	ATM:p.D2448N	Missense	-	0.17%	-
	DNMT3A	DNMT3A:p.R882H	Missense	-	0.23%	1.18%
	ERBB4	ERBB4:p.E233Q	Missense	-	0.48%	0.34%
	TP53	TP53:p.R158G	Missense	77.47%	-	-
CHE04	ALK	ALK:p.R806C	Missense	-	0.22%	-

0	BRAF	BRAF:p.R603*	Nonsense	-	0.03%	-
	KDR	KDR:p.Q1301Hfs*20	Frameshift	-	0.13%	-
	PIK3CA	PIK3CA:p.R115*	Nonsense	-	0.14%	-
	TP53	TP53:p.Y205H	Missense	37.35%	-	-
	TP53	TP53:p.L330P	Missense	19.18%	-	-
CHE045	ATM	ATM:p.R2719C	Missense	-	0.11%	-
	PTCH1	PTCH1:p.T1052M	Missense	-	0.11%	-
	TP53	TP53:p.Q38*	Nonsense	45.56%	-	-
	ATM	ATM:p.I2888T	Missense	26.13%	-	-
CHE047	ALK	ALK:p.R1181C	Missense	-	0.50%	-
	EGFR	EGFR:p.R803Q	Missense	-	0.12%	-
	EGFR	EGFR:p.R958H	Missense	-	0.14%	-
	PTCH1	PTCH1:p.R255Q	Missense	-	0.07%	-
	TP53	TP53:p.S241P	Missense	28.23%	-	-
	TP53	TP53:p.M237I	Missense	32.40%	-	-

CHE048	JAK2	JAK2:p.S605A	Missense	-	0.05%	-
	PTCH1	PTCH1:p.R603C	Missense	-	0.26%	-
	TP53	TP53:p.R306*	Nonsense	35.91%	-	-
CHE054	ALK	ALK.R1575H	Missense	-	0.44%	-
	ATM	ATM.E2444K	Missense	-	0.18%	-
	BRAF	BRAF.L597R	Missense	-	0.24%	-
	TP53	TP53.R114C	Missense	23.77%	-	-

VAF, variant allele frequency.

Supplementary Table 4. Emergence of novel TP53 mutations in ctDNA following chemotherapy.

ID	TRG	Pre-NCT TP53 ctDNA VAF	Pre-surg TP53 ctDNA VAF	Post-surg TP53 ctDNA VAF
CHNE008	2	2.52%	0.00%	0.00%
CHNE020	2	4.00%	0.00%	0.00%
CHNE042	3	0.00%	2.80%	2.72%
CHNE072	3	0.23%	1.70%	3.03%
CHNE234	1	7.18%	0.00%	0.00%
CHNE263	2	0.00%	0.00%	0.00%
CHNE323	2	0.00%	0.00%	0.00%
CHNE331	3	0.00%	1.94%	3.99%
CHNE445	1	5.03%	0.00%	0.00%

VAF, variant allele frequency.