

Figure S1. Amplification and expression analysis of TSTA3 in TCGA database. (A) Amplification and variation of TSTA3 gene in different cancers. **(B)** Expression level of TSTA3 in different cancers. Red represents the expression of TSTA3 in cancer tissue is higher than that of normal tissue. Blue represents the expression of TSTA3 in cancer tissue is lower than that of normal tissue. **(C)** In ovarian cancer, breast cancer, lung cancer and colon adenocarcinoma TSTA3 mRNA was dramatically upregulated in tumor tissues in comparison to that of non-tumor tissues in TCGA database.

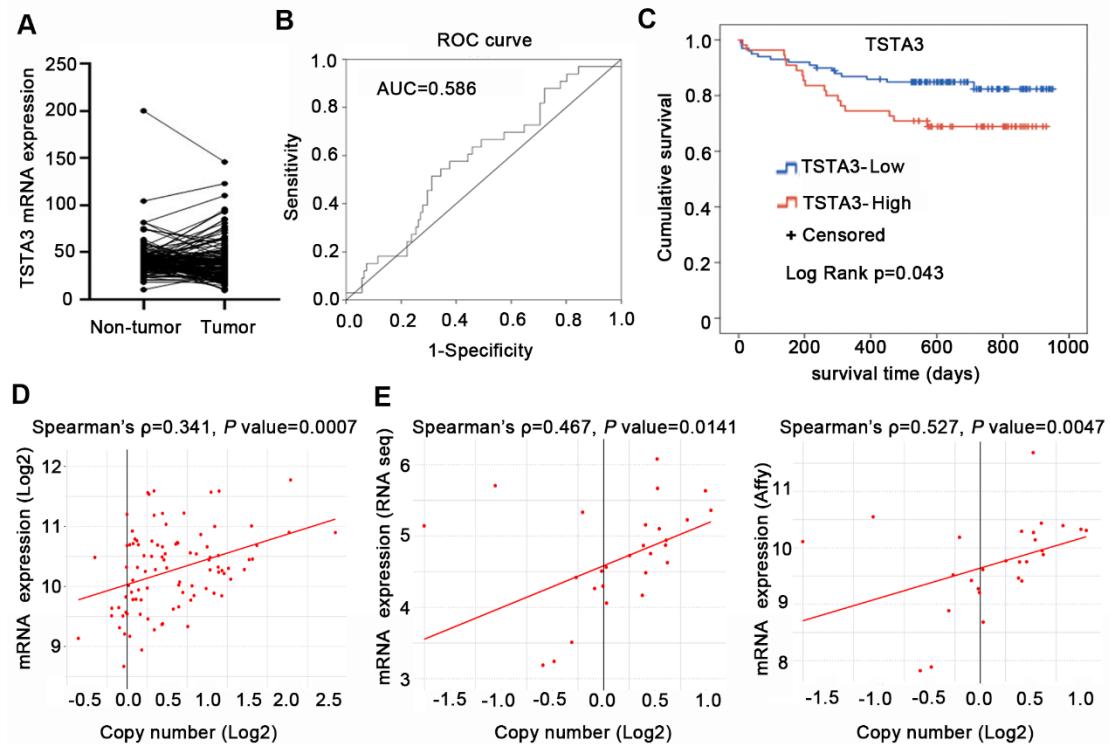


Figure S2. The expression analysis of TSTA3 in transcriptomic sequencing data of ESCC. (A) The expression level of TSTA3 mRNA in ESCC tissues compared with paired normal tissues from 155 cases. (B) ROC curve analyses of TSTA3 mRNA expression in RNA sequencing data of 155 ESCC cases ($AUC = 0.586, P < 0.001$). (C) Kaplan-Meier's analysis between ESCC patients with different TSTA3 mRNA expression in RNA sequencing data of 155 ESCC cases. (D) Correlation analysis between TSTA3 amplification and RNA expression in ESCC tissues of TCGA database. (E) Correlation analysis between TSTA3 amplification and RNA expression in 27 ESCC cell lines of Cancer Cell Line Encyclopedia (CCLE) database.

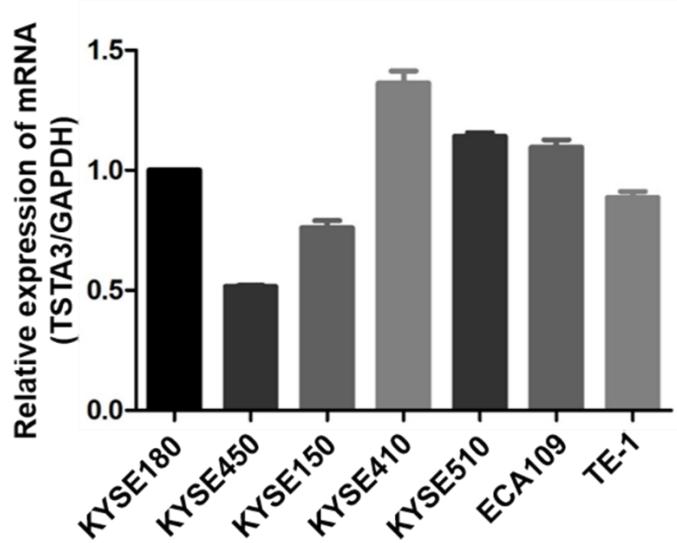


Figure S3. The mRNA expression pattern of TSTA3 in ESCC cell lines detected by RT-qPCR.

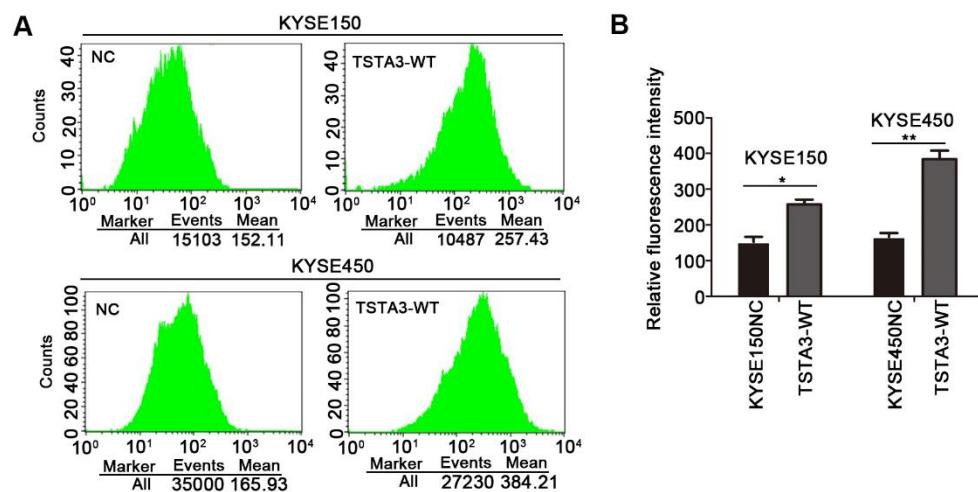


Figure S4. UEA-I fluorescence analysis of ESCC cells. **(A)** UEA-I fluorescence of KYSE150 (upper) and KYSE450 (bottom) cells stably overexpressing TSTA3 or control vector were analyzed by FACS. **(B)** The panel shows quantitation of fluorescence. All data are presented as the mean \pm standard deviation and three independent experiments. * P < 0.05, ** P < 0.01.

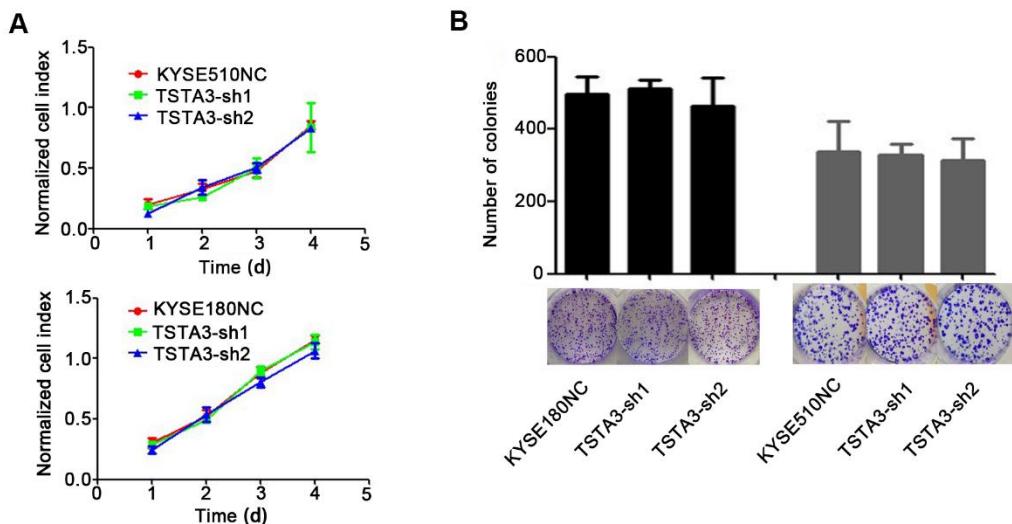


Figure S5. The effect of TSTA3 silencing on ESCC proliferation and colony formation. (A) Cell proliferation ability of KYSE510 (upper) and KYSE180 (bottom) cells with TSTA3 knockdown and negative control was analyzed by MTT. (B) The ability of colony formation was analyzed by colony formation assay. All data are presented as the mean \pm standard deviation and three independent experiments.

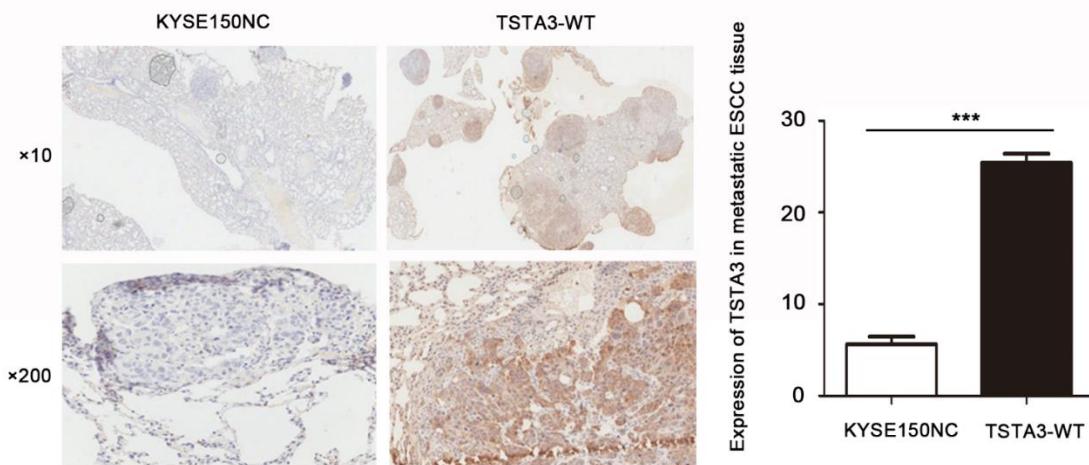


Figure S6. Representative immunohistochemistry images of TSTA3 expression in metastatic tumor nodules in the lung section of nude mice intravenously injected with KYSE150 cells stably overexpressing TSTA3 or control vector.

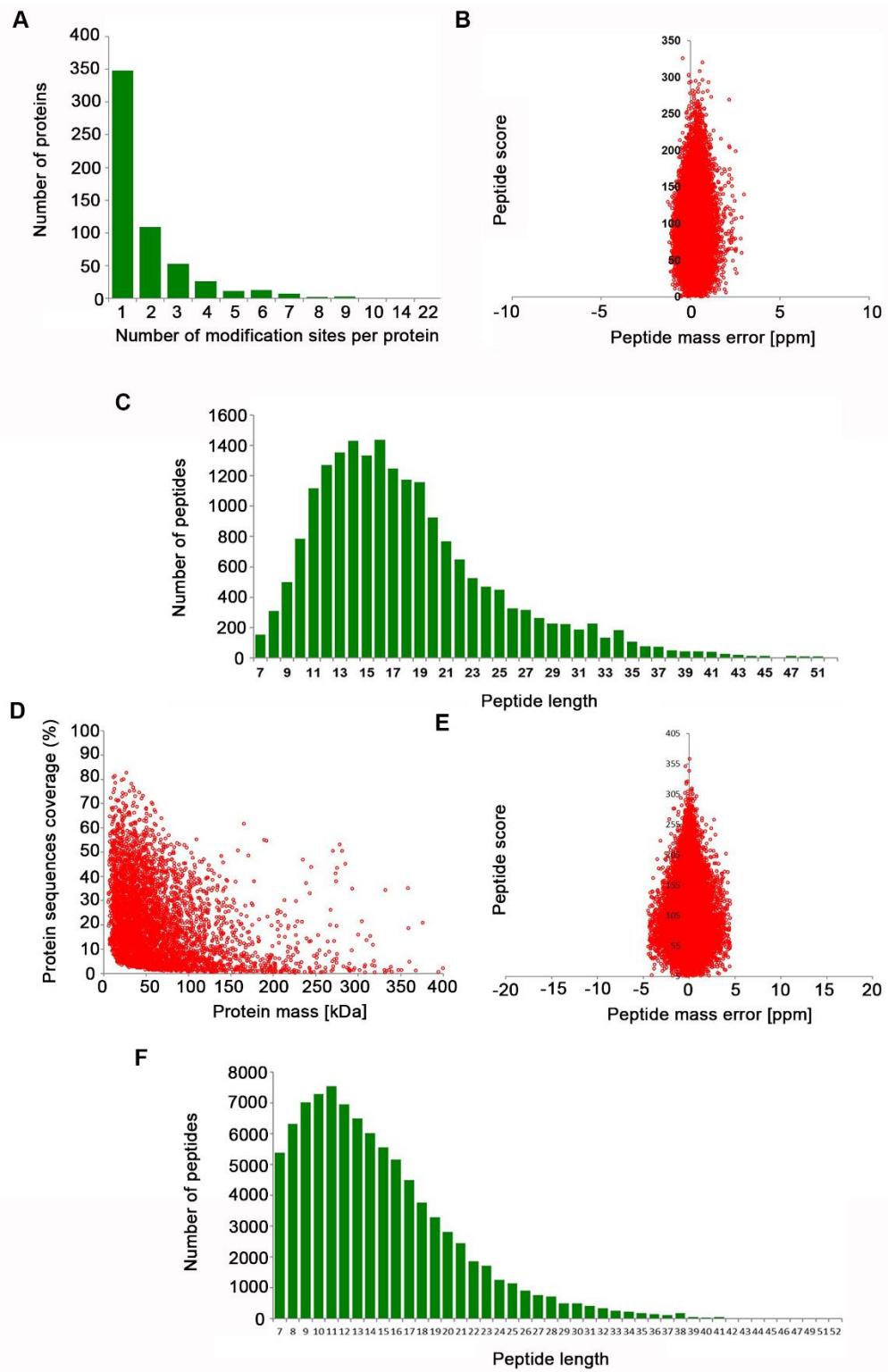


Figure S7. Quality control of N-glycoproteomics and proteomics analysis. **(A)** Statistic analysis of the number of modified site per glycosylated proteins. **(B)** The mass error of the whole identified peptides in N-glycoproteomics analysis. **(C)** Statistic analysis of the whole identified peptide length in N-glycoproteomics analysis. **(D)** The relationship between protein coverage and molecular weight in proteomics analysis. **(E)** The mass error of the whole identified peptides in proteomics analysis. **(F)** Statistic analysis of the whole identified peptide length in proteomics analysis.

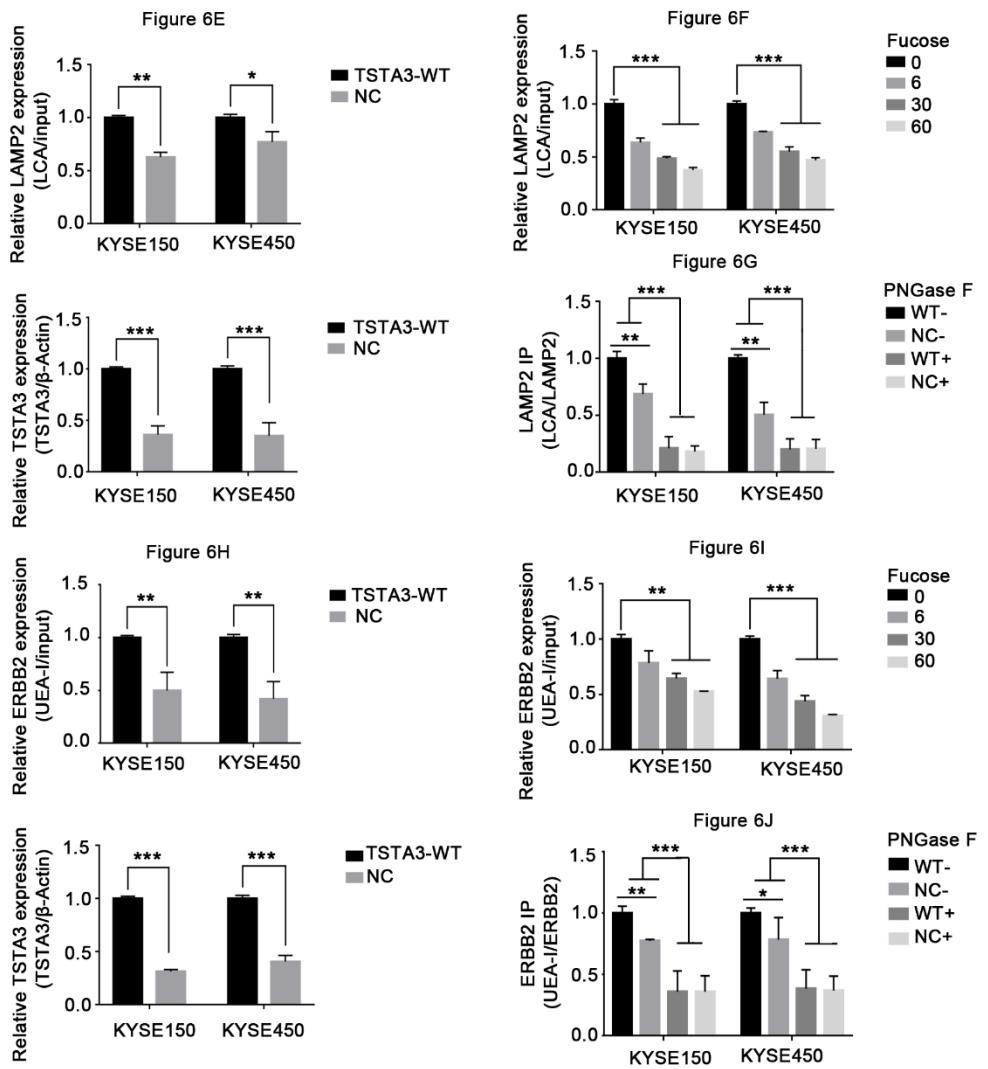


Figure S8. The densitometries analysis on the western blots of Figure 6E-6J. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

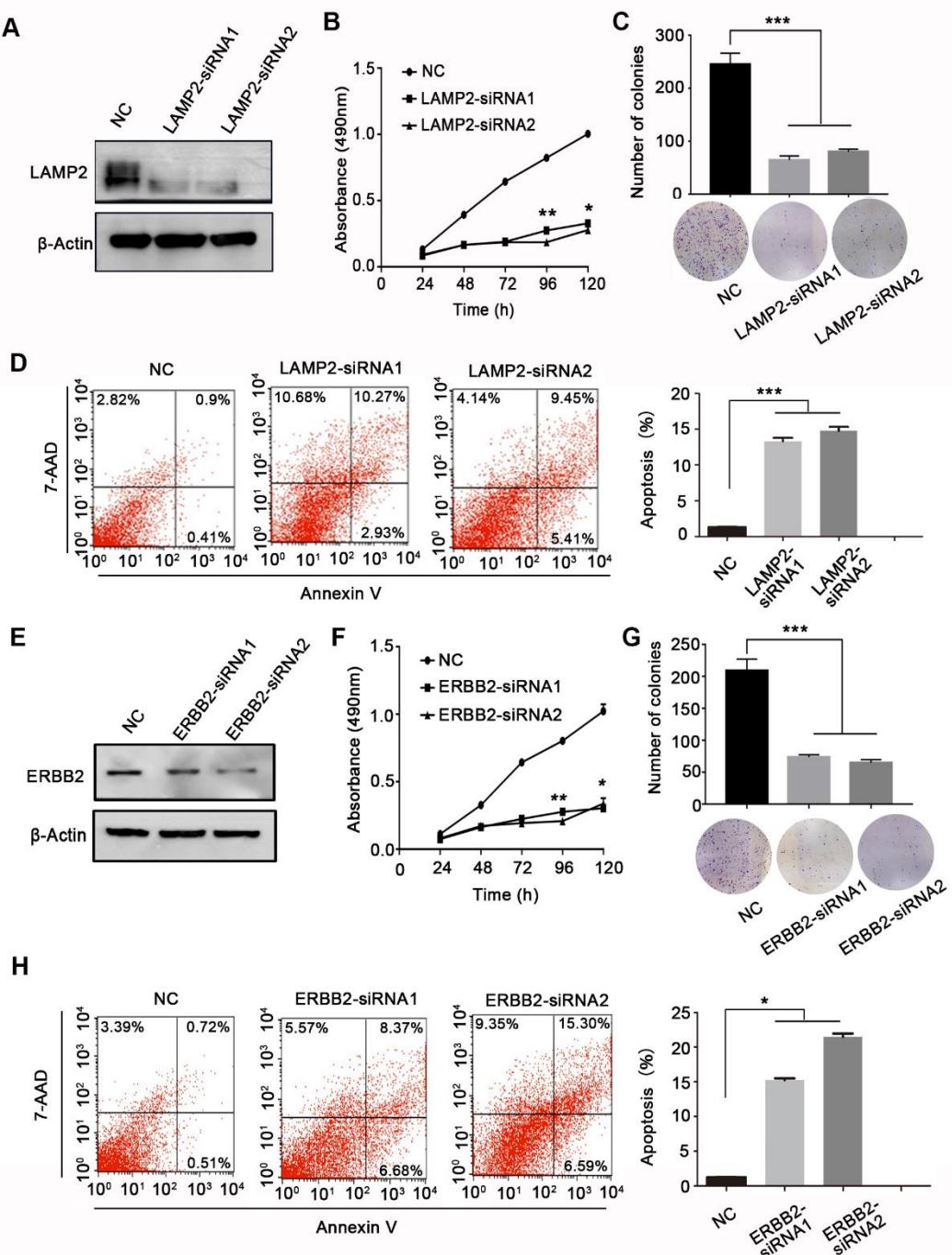


Figure S9. The effect of LAMP2 or ERBB2 knockdown on the proliferation, colony formation and apoptosis of KYSE150 ESCC cells. (A, E) Knockdown efficiency of LAMP2 or ERBB2 were tested by western blot. (B, F) LAMP2 or ERBB2 knockdown inhibited the proliferation of KYSE150 cells monitored by MTT assay. (C, G) The numbers of colony formation were decreased in LAMP2 or ERBB2 knockdown group compared to the control. (D, H) LAMP2 or ERBB2 knockdown increased apoptosis rate of KYSE 150 cells. Data represent the mean \pm SD. At least three independent experiments were performed in triplicate. Statistical analysis was performed with a two-sided t test. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

Table S1: Association of TSTA3 amplification with clinic-pathologic factors in 663 ESCC cases.

Clinical, epidemiological or pathological feature	Total(N)	TSTA3_WT	Proportion	TSTA3_AMP	Proportion	P value
All cases	663	438	66.06%	225	33.94%	
	<60	275	61.82%	105	38.18%	
Age	60-69	301	69.77%	91	30.23%	0.13178
	≥70	87	66.67%	29	33.33%	
Sex	Male	438	64.84%	154	35.16%	0.353595
	Female	225	68.44%	71	31.56%	
	Upper thoracic	34	50.00%	17	50.00%	
Tumor location	Middle thoracic	427	67.45%	139	32.55%	0.126173
	Lower thoracic	202	65.84%	69	34.16%	
	Grade 1	42	71.43%	12	28.57%	
Histological grade	Grade 2	395	64.81%	139	35.19%	0.619902
	Grade 3	226	67.26%	74	32.74%	
Pathologic Stage	I&II	383	70.76%	112	29.24%	0.002926
	III&IV	280	59.64%	113	40.36%	
Lymphatic.metastasis	Yes	283	59.36%	115	40.64%	0.001734
	No	380	71.05%	110	28.95%	
	Dead	251	65.74%	86	34.26%	
Prognosis (Log-rank Mantel-Cox test)	Survival	408	66.18%	138	33.82%	0.877426
	Missing	4	75.00%	1	25.00%	

Table S2: Association of TSTA3 mRNA expression with clinic-pathologic factors in 155 ESCC cases

Characteristics		Total (n=155)	TSTA3 expression		<i>P</i> value
			Low	High	
Age	<60	56	32(57.1)	24(42.9)	0.149
	≥60	99	68(68.7)	31(31.3)	
Sex	Female	52	34(65.4)	18(34.6)	0.872
	Male	103	66(64.1)	37(35.9)	
Tumor location	Upper and Middle	39	25(64.1)	14(35.9)	0.095
	Lower	116	75(64.7)	41(35.3)	
Histological grade	Grade 1	4	3(75.0)	1(25.0)	0.468
	Grade 2	105	67(63.8)	38(36.2)	
	Grade 3	42	26(61.9)	16(38.1)	
Pathologic Stage	Grade 4	4	4(100.0)	0(0.0)	0.067
	I&II	83	59(71.1)	24(28.9)	
	III&IV	72	41(56.9)	31(43.1)	
Pathologic T Stage	1+2	41	24(58.5)	17(41.5)	0.351
	3+4	114	76(66.7)	38(33.3)	
Lymphatic metastasis	No	82	58(70.7)	24(29.3)	0.087
	Yes	73	42(57.5)	31(42.5)	

Table S3. Clinical features of ESCC patients having metastatic lymph nodes samples

Sample ID	gender	Age at diagnosis	Tumor Grade	Location(Upper/Middle/Lower)	Smoking Status	Drinking Status	Pathological T	Pathological N	Clinical M	Stage(TNM)	Lymphatic metastasis	Lymph node	Survival status	Survival after diagnosis (months)
ESCC007	Female	66	G3	Middle	never	never	T3	N3	M0	IIIC	Yes	L1: left gastric node; L2: paracardial node; L3: pretracheal node	Deceased	8
ESCC009	Female	55	G2	Lower	never	never	T3	N3	M0	IIIC	Yes	L1: pretracheal node; L2: right recurrent laryngeal nerve node	Living	22
ESCC012	Male	65	G2	Middle	moderate	never	T3	N1	M0	IIIA	Yes	L1: pretracheal node; L2: middle paraesophageal node; L3: right recurrent laryngeal nerve node	Deceased	2
ESCC013	Male	64	G2-3	Middle	never	never	T3	N1	M0	IIIA	Yes	L1: left gastric node	Living	14
ESCC019	Male	69	G2-3	Middle	heavy	heavy	T2	N0	M0	IIIB	Yes	L1: subcarinal node 1; L2: subcarinal node 2; L3: left gastric node	Deceased	9
ESCC020	Male	63	G3	Middle	heavy	moderate	T3	N3	M0	IIIC	Yes	L1: subcarinal node; L2: middle paraesophageal node; L3: left gastric node	Deceased	12
ESCC022	Female	65	G2-3	Middle	moderate	never	T3	N3	M0	IIIC	Yes	L1: subcarinal node; L2: left recurrent laryngeal nerve node; L3: pretracheal node	Deceased	9
ESCC023	Male	72	G3	Lower	never	never	T3	N2	M0	IIIB	Yes	L1: paracardial node 1; L2: paracardial node 3; L3: paracardial node 4	Living	16
ESCC030	Male	52	G3	Middle	never	never	T3	N1	M0	IIIA	Yes	L1: left gastric node	Living	14
ESCC035	Female	62	G2	Middle	never	never	T3	N2	M0	IIIB	Yes	L1: splenic node; L2: left gastric node; L3: common hepatic node	Deceased	11
ESCC036	Female	58	G2	Middle	never	never	T3	N2	M0	IIIB	Yes	L1: middle paraesophageal node; L2: right recurrent laryngeal nerve node; L3: lower paraesophageal node	Deceased	7

Table S5. Primers and siRNA used in this study.

Primer Name	Forward Primer Sequence (5' to 3')	Reverse Primer Sequence (5' to 3')	Used for
TSTA3-primer 1	5'-GCGGATTCTAGTGACAGGGG	GATGGATG ACGTGTGT GGT	RT-PCR for TSTA3 expression analysis
GAPDH-primer 2	CGGAGTCAACGGATTGGTCGTAT	AGCCTTC TCCATGGTGGTGAAGAC	RT-PCR for GAPDH expression analysis
TSTA3-sh1	CDS:CCGGCCCGAATATCAAATACAATTCTCGAGAAAT TGATTTGAT ATTCGG A TTTTG		TSTA3 Knockdown
TSTA3-sh2	UTR:CCGGAGCTGGAAAGACAGGATCAGGTCTCGAGACCTGATCCTGTCT TCCAGCTTTTG		TSTA3 Knockdown
LAMP2-siRNA1	CTGGGATGTTCTTGACAA		LAMP2 Knockdown
LAMP2-siRNA2	CCACTTGCCTTATGCAA		LAMP2 Knockdown
LAMP2-siRNA3	GCGGTCTTATGCATTGGAA		LAMP2 Knockdown
ERBB2-siRNA1	GGAGACCCGCTGAACAATA		ERBB2 Knockdown
ERBB2-siRNA2	CAGACACGTTGAGTCCAT		ERBB2 Knockdown
ERBB2-siRNA3	GGAAGGACATCTTCCACAA		ERBB2 Knockdown