

Supplementary

Table S1 List of esophageal squamous cell carcinoma information

Dataset	TCGA-ESCC	GSE20347	ESCC dataset
Platform species	TCGA	GPL571	–
	Homo sapiens	Homo sapiens	Homo sapiens
	Esophageal	Esophageal	Esophageal
Tissue	Squamous cell	Squamous cell	Squamous cell
	Carcinoma tumor	Carcinoma tumor	Carcinoma tumor
	Tissues	Tissues	Tissues
Samples in normal group	11	17	6
Samples in tumor group	163	17 Genome wide analysis of DNA copy number neutral loss of heterozygosity (CNNLOH) and its relation to gene expression in esophageal squamous cell carcinoma	6
Reference	–	–	–

TCGA, The Cancer Genome Atlas; ESCC, esophageal squamous cell carcinoma.

Table S2 Ubiquitination related genes list

DCUN1D5	USP35	LEO1	MID2	TPP2	TUFM
HCK	RNF128	LGR4	USPL1	TRAF1	DDX5
TRAIP	UBR7	LGR5	ALS2	TRAF2	TRIM34
UHFR1	IKBKG	LGR6	STAM2	TRAF3	IVNS1ABP
TRIM6	HSPA4	LMO7	FIG4	TRAF3IP2	RPS2
KLHL35	HSPA1A	LNPEP	KCTD10	TRAF4	AR
FBXL8	BIRC3	LNX1	KLHL12	TRAF5	KCTD17
ANKRD13B	SOD1	LONRF1	MAP1LC3A	TRAF6	H2AC4
ISG15	USP31	LRP5	TRIM26	TRAF7	STAT3
SOC3S	FAU	LRP6	KLHDC2	TRIM11	DDX17
KBTBD12	HECTD4	LRR1	ARRB1	TRIM13	MAGED1
AMFR	TSG101	LRRC41	NSMCE1	TRIM17	RPA1
ANAPC1	MAPT	LRRK2	JUN	TRIM21	SMAD1
ANAPC10	PINK1	LRSAM1	ZSWIM2	TRIM25	PACRG
ANAPC11	UBL7	LTN1	KLHL15	TRIM27	GID8
ANAPC13	USP29	MAGEL2	PTTG1	TRIM31	EPRS1
ANAPC2	GSK3B	MAN1A1	GAPDH	TRIM32	CCT3
ANAPC4	MAEA	MAN1B1	DAXX	TRIM36	CCT4
ANAPC5	RING1	MARCHF5	TAB2	TRIM37	DLEU2
ANAPC7	USP43	MARCHF6	TUBB	TRIM38	RPS7
ANKZF1	SPOP	MARCHF7	BIRC7	TRIM39	CCT6A
AREL1	TRIM28	MDM2	NOSIP	TRIM4	SLC25A5
ARIH2	RNF167	MEX3C	CDK5	TRIM41	GLE1
ASB1	USP39	MGRN1	G3BP1	TRIM44	MAPK1
ASB10	UBFD1	MIB2	TRIML1	TRIM5	KLHDC1
ASB11	SNCA	MIR138-1	HSPD1	TRIM50	PDCD6IP
ASB12	MARCHF4	MKRN1	C10orf90	TRIM52	USP17L4
ASB13	RAD23B	MTA1	INSIG1	TRIM56	SENP3
ASB14	PEL12	MYLIP	GABARAPL2	TRIM58	UBL4B
ASB15	RFWD3	NAE1	SIRT1	TRIM62	WDR77
ASB16	EGFR	NCCRP1	PAN2	TRIM63	MOCS3
ASB17	UBE2QL1	NEDD4	KCTD13	TRIM68	MAP1LC3C
ASB18	DTX1	NEDD4L	EP300	TRIM69	XPO1
ASB2	UFM1	NFE2L2	HNRNPU	TRIM71	H2AX
ASB3	RMND5A	NFX1	SPRTN	TRIM9	YWHAZ
ASB4	USP51	NMI	MALT1	TRIP12	SPART
ASB5	LOC100532749	NOD2	MAVS	TTC3	TP53BP1
ASB6	MATR3	NPEPPS	TNF	UBA1	H2AC18
ASB7	HIF1A	NPLOC4	UBE2DNL	UBA3	VDAC1
ASB8	USP42	OBI1	CCNE1	UBA5	ILF3
ASB9	PRNP	OS9	MKRN4P	UBA52	ACTB
ATG7	UFC1	OTUB1	NSFL1C	UBA6	BUB1B
AUP1	MARCHF3	OTUB2	CCT2	UBA7	WRNIP1
BABAM2	HDAC6	OTULIN	SMAD3	UBAC1	IQU8
BAG6	PARP1	PAF1	YWHAQ	UBB	CHEK1
BARD1	CAND1	PARP10	TAF15	UBC	CCAR2
BCAP31	ARK2C	PCNA	DDI2	UBE2A	RPS8
BCL10	MARCHF10	PEL11	KLHDC10	UBE2B	ELAVL1
BFAR	DCUN1D1	PEL13	UBE2L1	UBE2C	UNC13A
BIRC2	SUMO4	PEX10	WDR48	UBE2D1	E4F1
BLMH	UBASH3B	PEX12	SPOPL	UBE2D2	UBQLN3
BRCA1	PML	PEX13	ANKRD13A	UBE2D3	LMNB1
BRCA2	USP17L24	PEX14	TUBA1B	UBE2D4	DCAF15
BRCC3	PFN1	PEX2	LOC107303340	UBE2E1	MAD2L1
BTBD1	NUB1	PEX5	UBAC2	UBE2E2	CFTR
BTBD6	UBL5	PJA1	VPS36	UBE2E3	KCTD5
BTRC	RNF169	PJA2	EPM2A	UBE2F	MAP3K5
CALR	RIG1	PLAA	DDA1	UBE2G1	CASP3
CALR3	NEURL1	PP1A	TRIM45	UBE2G2	ACLY
CANX	HSPA1B	PRKDC	N4BP1	UBE2H	HNRNPL
CAV1	NEURL3	PRKN	NDFIP2	UBE2J1	MYH9
CBFB	USP45	PRPF19	XRC55	UBE2J2	KLHDC3
CBL	USP17L25	PSMA1	JADE2	UBE2K	RPA2
CBLB	USP17L29	PSMA2	PCPB2	UBE2L3	RYBP
CBL2	USP17L30	PSMA3	COPS4	UBE2L5	PRMT1
CCDC47	USP17L26	PSMA4	SIAH3	UBE2L6	MALAT1
CCNF	USP17L27	PSMA5	NEURL4	UBE2M	STAT1
CDC16	USP17L28	PSMA6	DCAF5	UBE2N	SPSB3
CDC20	DCTN1	PSMA7	CASP8	UBE2NL	LMNA
CDC23	DTX2	PSMA8	IRF2BPL	UBE2O	DCAF16
CDC26	CDKN1B	PSMB1	RNF113A	UBE2Q1	GRB2
CDC27	USP54	PSMB10	MIR7-3HG	UBE2Q2	SYNCRIP
CDC34	USP53	PSMB11	HTT	UBE2R2	ZFAND2B
CDC73	MID1	PSMB2	ASCC2	UBE2S	TRIM40
CLGN	VAPB	PSMB3	NPM1	UBE2T	FLNA
CNOT4	NFKBIA	PSMB4	TRIM3	UBE2U	COPS7A
CTR9	RNF166	PSMB5	RABGEF1	UBE2V1	COPS2
CUL1	MARCHF9	PSMB6	CHCHD10	UBE2V2	ZFAND1
CUL2	UBAP2L	PSMB7	SGK1	UBE2W	HSPA1L
CUL3	USP27X	PSMB8	IRAK1	UBE2Z	PRKAR1A
CUL5	UBTD1	PSMB9	COPS8	UBE3A	CCT7
CUL7	SAE1	PSMC1	HDAC1	UBE3B	PGAM5
DCAF1	ZRANB1	PSMC2	BUB3	UBE3C	CERNA3
DDB2	STAM	PSMC3	TCP1	UBE3D	AKTIP
DDX3X	UBAP2	PSMC4	PPARGC1A	UBE4A	CALCOCO2
DERL1	DTX4	PSMC5	NCL	UBE4B	MRE11
DERL2	MKRN2	PSMC6	HSPBP1	UBOX5	RPS3A
DERL3	DTX3	PSMD1	CLTC	UBQLN1	DCAF10
DET1	UBXN7	PSMD10	CSNK2A1	UBQLN2	AURKB
DNAJB2	NEURL1B	PSMD11	MDM4	UBR1	TUBB2A
DNAJB9	USP17L6P	PSMD12	SIGMAR1	UBR2	SMAD4
DNAJC10	HNRNPA2B1	PSMD13	HSPB1	UBR4	MCM7
DTX3L	PPIL2	PSMD14	TRIM48	UBR5	SLC25A13
DZIP3	USP41P	PSMD2	POLR2A	UBXN10	GRWD1
ECPAS	CCNB1IP1	PSMD3	TOM1	UBXN4	UBXN6
EDEM1	ATXN2	PSMD4	SMAD2	UBXN8	AIFM1
EDEM2	EPS15	PSMD5	PKM	UCHL3	ABRAXAS1
EDEM3	RNF170	PSMD6	RPS20	UFD1	TAB1
ELOB	LOC125177489	PSMD7	RB1	UFL1	RPLP0
ELOC	STAMBP	PSMD8	TIA1	UHRF2	OTUD3
ERCC8	AMBRA1	PSMD9	FANCD2	UMOD	DNAJA2
ERLEC1	CDKN1A	PSME1	RELA	UNKL	HSPA6
ERLIN1	BRAP	PSME2	CDK2	USP14	SENP6
ERLIN2	NDFIP1	PSME3	RPN1	USP19	E2F1
FAF1	CDKN2A	PSME4	ANG	USP5	DNM1L
FAF2	SETX	PSMF1	DHX9	USP7	RAB7A
FAM8A1	ZUP1	PTPN22	DDRKG1	USP8	AIMP2
FBXL12	RIPK1	RAD18	RANBP9	USP9X	PON2
FBXL13	TRIM2	RAD51	IRF2BP1	VCP	RBBP4
FBXL14	TRIM65	RAG1	TRIM59	VHL	CDT1
FBXL15	KCMF1	RBBP6	RC3H1	WAC	MED8
FBXL16	AKT1	RBCK1	TRIM35	WFS1	NONO
FBXL18	USP50	RBX1	SRC	WNT1	GPS1
FBXL19	RNF180	RCHY1	RNF145	WNT3A	DCUN1D5
FBXL20	UBXN1	RFPL	DIABLO	WSB1	RHOA
FBXL22	USP17L5	RHBDD2	RPL11	WWP1	NEAT1
FBXL3	PTEN	RLIM	BAG2	WWP2	VCP1P1
FBXL4	MARCHF11	RNF10	WDR26	XIAP	UBE2L2
FBXL5	COPS5	RNF103	HNRNPC	YOD1	ANAPC16
FBXL7	ZNF598	RNF11	PTBP1	ZBTB16	GPR37
FBXO10	CHMP2B	RNF111	HNRNPA3	ZFP91	FBXO42
FBXO11	PIAS4	RNF112	BECN1	ZNRF1	EGF
FBXO15	UBL3	RNF114	KLHL8	ZNRF2	YBX1
FBXO17	FBXO3	RNF115	PHGDH	ZNRF3	ATG5
FBXO2	TRIM8	RNF121	PABPC1	ZYG11B	RUVBL1
FBXO21	RNF149	RNF122	CCT5	UCHL1	CSNK2B
FBXO22	RPS3	RNF123	ESR1	UBE2I	KLHL7
FBXO27	ERBB4	RNF126	KPNB1	SQSTM1	LOC109504728
FBXO30	IKBKB	RNF13	TRIM15	SUMO1	EIF3F
FBXO31	RMND5B	RNF130	TAX1BP1	TP53	RIPK2
FBXO32	HSP90AB1	RNF133	SFPQ	USP2	IGF2BP3
FBXO38	UBASH3A	RNF135	SOC52	ARIH1	RPS6
FBXO4	BMI1	RNF138	USP17L9P	USP15	NLRP3
FBXO40	CUL9	RNF14	OGT	USP4	H2BC21
FBXO41	UBTD2	RNF141	PIN1	USP1	EIF3H
FBXO44	ZNRF4	RNF144A	LRPPRC	USP11	OTUD1
FBXO6	C9orf72	RNF144B	USP17L7	COP1	ABL1
FBXO7	MYC	RNF146	H2AC20	USP25	RPS11
FBXO9	NEURL2	RNF152	TNFAIP1	USP21</td	

Table S3 Clinical data for qRT-PCR of patients with esophageal squamous cell carcinoma in our institution

Characteristic	Levels	Overall, n (%)
Gender, n (%)	Female	6 (42.9)
	Male	8 (57.1)
Age (years), n (%)	≤60	5 (35.7)
	>60	9 (64.3)
Smoking, n (%)	Yes	4 (28.6)
	No	10 (71.4)
Pathologic T stage, n (%)	1b	2 (14.3)
	2	1 (7.1)
	3	11 (78.6)
Pathologic N stage, n (%)	0	8 (57.1)
	1	6 (42.9)
Pathologic TNM stage, n (%)	I	3 (21.4)
	II	4 (28.6)
	III	7 (50.0)

qRT-PCR, quantitative real-time polymerase chain reaction; TNM, tumor-node-metastasis.

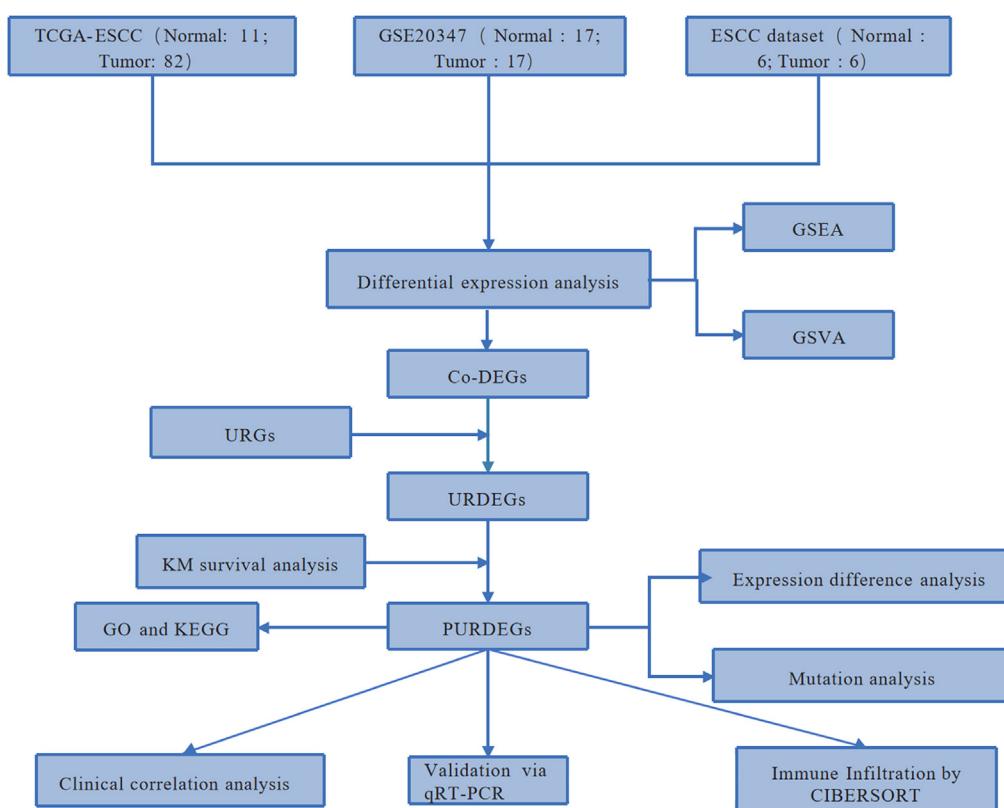


Figure S1 The flow chart of this study. TCGA, The Cancer Genome Atlas; ESCC, esophageal squamous cell carcinoma; GSEA, gene set enrichment analysis; GSVA, gene set variation analysis; DEGs, differentially expressed genes; Co-DEGs, common differentially expressed genes; URGs, ubiquitination related genes; URDEGs, ubiquitination related differentially expressed genes; KM, Kaplan-Meier; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; PURDEGs, prognostic ubiquitination related differentially expressed genes; qRT-PCR, quantitative real-time polymerase chain reaction.

Table S4 Differential expression of prognostic ubiquitination related differentially expressed genes between tumor and normal group

Gene symbol	logFC	Ave Expr	t	P value	adj.P. Val	B
<i>BUB1B</i>	2.907638411	10.67708556	9.701416664	8.98E-16	2.95E-13	25.46130842
<i>CHEK1</i>	2.148592332	10.43675454	8.596484416	1.93E-13	2.67E-11	20.21561398
<i>DNMT1</i>	1.322419148	13.03038172	7.313922564	9.05E-11	5.76E-09	14.21604755
<i>IRAK1</i>	0.786297048	13.51830963	4.033940748	0.000112564	0.001072582	0.673721035
<i>PRKDC</i>	1.023552163	14.82373262	4.435063778	2.53E-05	0.000300617	2.091398329

FC, fold change.

Table S5 GO enrichment analysis results of PURDEGs

Ontology	ID	Description	Gene ratio	Bg ratio	P value	p.adjust
BP	GO:0007093	Mitotic cell cycle checkpoint signaling	3/5	130/18,800	0.000003	0.0007
BP	GO:0000075	Cell cycle checkpoint signaling	3/5	170/18,800	7. 17e-06	0.0007
BP	GO:0048660	Regulation of smooth muscle cell proliferation	3/5	170/18,800	7. 17e-06	0.0007
BP	GO:0048659	Smooth muscle cell proliferation	3/5	173/18,800	0.000008	0.0007
BP	GO:1901991	Negative regulation of mitotic cell cycle phase transition	3/5	183/18,800	0.000009	0.0007
CC	GO:0098687	Chromosomal region	4/5	366/19,594	0.000001	0.000015
CC	GO:0005657	Replication fork	2/5	60/19,594	9. 17e-05	0.0012
CC	GO:0000781	Chromosome, telomeric region	2/5	166/19,594	0.0007	0.0061
CC	GO:0000775	Chromosome, centromeric region	2/5	227/19,594	0.0013	0.0085
CC	GO:0000793	Condensed chromosome	2/5	255/19,594	0.0016	0.0085
MF	GO:0106310	Protein serine kinase activity	4/5	360/18,410	0.000001	0.000009
MF	GO:0004674	Protein serine/threonine kinase activity	4/5	430/18,410	0.000001	0.000009
MF	GO:0004712	Protein	4/5	446/18,410	0.000002	0.000009
MF	GO:0034511	Serine/threonine/tyrosine kinase activity U3 snoRNA binding	1/5	11/18,410	0.0030	0.0119
MF	GO:0035173	Histone kinase activity	1/5	16/18,410	0.0043	0.0139

PURDEGs, prognostic ubiquitination related differentially expressed genes; GO, Gene Ontology; BP, biological process; CC, cellular component; MF, molecular function.

Table S6 KEGG enrichment analysis results of PURDEGs

Ontology	ID	Description	Gene ratio	Bg ratio	P value	p.adjust
KEGG	hsa04110	Cell cycle	3/5	126/8,164	3.5098E-05	0.000982743
KEGG	hsa05170	Human immunodeficiency virus 1 infection	2/5	212/8,164	0.006373402	0.055568846
KEGG	hsa05166	Human T-cell leukemia virus 1 infection	2/5	222/8,164	0.006973003	0.055568846
KEGG	hsa03450	Non-homologous end-joining	1/5	13/8,164	0.007938407	0.055568846

PURDEGs, prognostic ubiquitination related differentially expressed genes; KEGG, Kyoto Encyclopedia of Genes and Genomes.

Table S7 GSEA enrichment analysis results of tumoral and normal groups of TCGA-ESCC

Description	Set size	Enrichment score	NES	p.adjust	qvalue
REACTOME_CELLULAR_SENESCENCE	195	0.668019316	2.395951801	0.014247622	0.010174749
REACTOME_OXIDATIVE_STRESS_INDUCE_D_SENESCENCE	123	0.696800107	2.345050889	0.014247622	0.010174749
REACTOME_PRE_NOTCH_EXPRESSION_AND_PROCESSING	118	0.654522734	2.195587763	0.014247622	0.010174749
REACTOME_TCF_DEPENDENT_SIGNALING_IN_RESPONSE_TO_WNT	233	0.571148166	2.082647255	0.014247622	0.010174749
REACTOME_SIGNALING_BY_NOTCH	245	0.548414986	2.014487256	0.014247622	0.010174749
REACTOME_TP53_REGULATES_TRANSLATION_OF_CELL_CYCLE_GENES	49	0.681456451	2.00442273	0.014247622	0.010174749
REACTOME_INTERLEUKIN_10_SIGNALING	45	0.676151277	1.975950827	0.014247622	0.010174749
REACTOME_SIGNALING_BY_WNT	330	0.518629621	1.957401346	0.014247622	0.010174749
REACTOME_STABILIZATION_OF_P53	57	0.644455771	1.954168478	0.014247622	0.010174749
REACTOME_WNT_LIGAND_BIOGENESIS_AND_TRAFFICKING	26	0.749982538	1.930660083	0.014247622	0.010174749

TCF, T-cell factor; WNT, wingless/integrated.

Table S8 GSVA enrichment analysis results of URDEGs prognosis model tumor and normal risk groups

Description	logFC	AveExpr	t	adj.P.Val
HALLMARK_E2F_TARGETS	0.715250974	0.005922015	6.663217808	4.70E-08
HALLMARK_G2M_CHECKPOINT	0.626121457	-0.005201528	6.205479127	2.17E-07
HALLMARK_MYC_TARGETS_V1	0.56733523	0.005722706	6.050748871	3.01E-07
HALLMARK_BILE_ACID_METABOLISM	-0.412377842	-0.060179788	-5.912231578	4.32E-07
HALLMARK_PANCREAS_BETA_CELLS	-0.430811065	0.029980397	-5.61252547	1.38E-06
HALLMARKADIPOGENESIS	-0.369245599	-0.059581948	-5.332512748	4.03E-06
HALLMARK_MTORC1_SIGNALING	0.405218098	-0.024026433	4.967454284	1.68E-05
HALLMARK_MYC_TARGETS_V2	0.540908834	-0.012001333	4.690428019	4.68E-05
HALLMARK_DNA_REPAIR	0.354561843	-0.042623382	4.459605339	0.000105904
HALLMARK_PEROXISOME	-0.296549989	-0.046264627	-4.230267452	0.000233988
HALLMARK_FATTY_ACID_METABOLISM	-0.319688099	-0.033592029	-4.136935958	0.000303898
HALLMARK_UV_RESPONSE_UP	0.291391452	-0.024281726	3.973360062	0.000514032
HALLMARK_SPERMATOGENESIS	0.296950787	-0.007393007	3.925276006	0.000566345
HALLMARK_WNT_BETA_CATENIN_SIGNALING	0.339263235	-0.035293326	3.771394339	0.000917534
HALLMARKMITOTIC_SPINDLE	0.314066961	-0.039807688	3.656199698	0.001286231
HALLMARKGLYCOLYSIS	0.25741932	-0.048561834	3.636258871	0.001292681
HALLMARKPI3K_AKT_MTOR_SIGNALING	0.261568269	-0.049291564	3.577807912	0.001489486
HALLMARKHEME_METABOLISM	-0.236748901	-0.043795934	-3.463810697	0.002073796
HALLMARKINTERFERON_ALPHA_RESPONSE	0.416675633	-0.01557065	3.221445357	0.00414287
HALLMARKOXIDATIVE_PHOSPHORYLATION	-0.317739401	-0.046325524	-3.220991155	0.00414287
HALLMARKINFLAMMATORY_RESPONSE	0.297849462	-0.020465011	3.10187554	0.005616579
HALLMARKTNFA_SIGNALING_VIA_NFKB	0.31192404	-0.016651817	3.0941393	0.005616579
HALLMARKINTERFERON_GAMMA_RESPONSE	0.345313766	-0.036729612	3.068614261	0.005616579
HALLMARKAPICAL_SURFACE	0.256926412	-0.000980826	3.066295728	0.005616579
HALLMARKXENOBIOTIC_METABOLISM	-0.225905587	-0.026024	-2.98285691	0.006961165
HALLMARKEPITHELIAL_MESENCHYMAL_TRANSITION	0.331898681	-0.006883818	2.827577767	0.010624406
HALLMARKPROTEIN_SECRETION	-0.24499224	-0.029441548	-2.800470457	0.011070349
HALLMARKUNFOLDED_PROTEIN_RESPONSE	0.209889854	-0.054357599	2.720773889	0.013418252
HALLMARKAPOPTOSIS	0.198782214	-0.052099091	2.588707565	0.01863225
HALLMARKP53_PATHWAY	0.201237959	-0.036210812	2.578250258	0.01863225
HALLMARKALLOGRAFT_REJECTION	0.261823745	-0.022972176	2.407728118	0.028428058
HALLMARKANDROGEN_RESPONSE	-0.194330611	-0.024039935	-2.293487465	0.036902273
HALLMARKNOTCH_SIGNALING	0.196369181	-0.050643814	2.182980653	0.047042472
HALLMARKAPICAL_JUNCTION	0.184787725	-0.024779512	2.14771485	0.049725165

GSVA, Gene Set Variation Analysis; URDEGs, Ubiquitination Related Differentially Expressed Genes; FC, Fold Change.

Table S9 COX regression to hub genes associated with OS in TCGA-ESCC

Characteristics	Total (N=82)	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
<i>BUB1B</i>					
Low	41	Reference		Reference	
High	41	0.464 (0.205–1.048)	0.065	0.925 (0.288–2.966)	0.896
<i>CHEK1</i>					
Low	41	Reference		Reference	
High	41	0.234 (0.091–0.601)	0.003	0.385 (0.112–1.318)	0.128
<i>DNMT1</i>					
Low	41	Reference		Reference	
High	41	0.260 (0.106–0.637)	0.003	0.395 (0.118–1.317)	0.130
<i>IRAK1</i>					
Low	41	Reference		Reference	
High	41	2.622 (1.136–6.052)	0.024	4.101 (1.652–10.183)	0.002
<i>PRKDC</i>					
Low	41	Reference		Reference	
High	41	0.347 (0.145–0.830)	0.017	0.785 (0.256–2.404)	0.672

OS, overall survival; TCGA, The Cancer Genome Atlas; ESCC, esophageal squamous cell carcinoma.