

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 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.

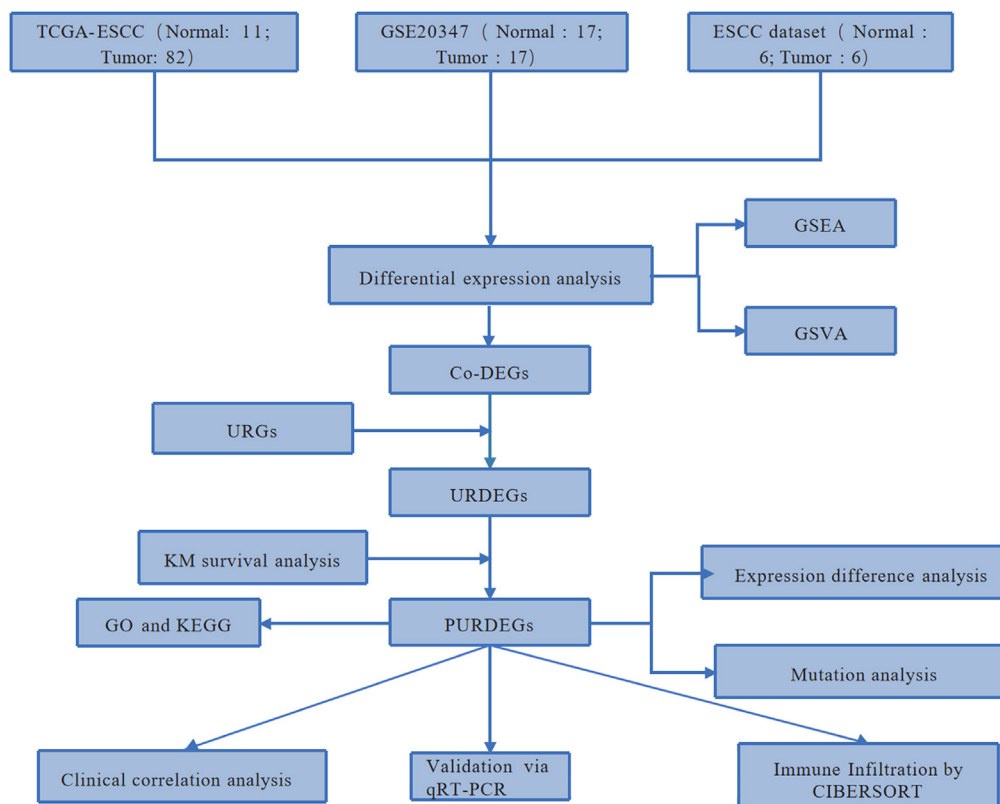


Figure S1 The flow chart of this study. TCGA, The Cancer Genome Atlas; ESCC, esophageal squamous cell carcinoma; GSEA, gene set enrichment analysis; GSVA, gene set variation analysis; DEGs, differentially expressed genes; Co-DEGs, common differentially expressed genes; URGs, ubiquitination related genes; 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_INDUCED_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_TRANSCRIPTION_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 |
| HALLMARK_ADIPOGENESIS | -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 |
| HALLMARK_MITOTIC_SPINDLE | 0.314066961 | -0.039807688 | 3.656199698 | 0.001286231 |
| HALLMARK_GLYCOLYSIS | 0.25741932 | -0.048561834 | 3.636258871 | 0.001292681 |
| HALLMARK_PI3K_AKT_MTOR_SIGNALING | 0.261568269 | -0.049291564 | 3.577807912 | 0.001489486 |
| HALLMARK_HEME_METABOLISM | -0.236748901 | -0.043795934 | -3.463810697 | 0.002073796 |
| HALLMARK_INTERFERON_ALPHA_RESPONSE | 0.416675633 | -0.01557065 | 3.221445357 | 0.00414287 |
| HALLMARK_OXIDATIVE_PHOSPHORYLATION | -0.317739401 | -0.046325524 | -3.220991155 | 0.00414287 |
| HALLMARK_INFLAMMATORY_RESPONSE | 0.297849462 | -0.020465011 | 3.10187554 | 0.005616579 |
| HALLMARK_TNFA_SIGNALING_VIA_NFKB | 0.31192404 | -0.016651817 | 3.0941393 | 0.005616579 |
| HALLMARK_INTERFERON_GAMMA_RESPONSE | 0.345313766 | -0.036729612 | 3.068614261 | 0.005616579 |
| HALLMARK_APICAL_SURFACE | 0.256926412 | -0.000980826 | 3.066295728 | 0.005616579 |
| HALLMARK_XENOBIOTIC_METABOLISM | -0.225905587 | -0.026024 | -2.98285691 | 0.006961165 |
| HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION | 0.331898681 | -0.006883818 | 2.827577767 | 0.010624406 |
| HALLMARK_PROTEIN_SECRETION | -0.24499224 | -0.029441548 | -2.800470457 | 0.011070349 |
| HALLMARK_UNFOLDED_PROTEIN_RESPONSE | 0.209889854 | -0.054357599 | 2.720773889 | 0.013418252 |
| HALLMARK_APOPTOSIS | 0.198782214 | -0.052099091 | 2.588707565 | 0.01863225 |
| HALLMARK_P53_PATHWAY | 0.201237959 | -0.036210812 | 2.578250258 | 0.01863225 |
| HALLMARK_ALLOGRAFT_REJECTION | 0.261823745 | -0.022972176 | 2.407728118 | 0.028428058 |
| HALLMARK_ANDROGEN_RESPONSE | -0.194330611 | -0.024039935 | -2.293487465 | 0.036902273 |
| HALLMARK_NOTCH_SIGNALING | 0.196369181 | -0.050643814 | 2.182980653 | 0.047042472 |
| HALLMARK_APICAL_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 |
| PRKDC | | | | | |
| 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.