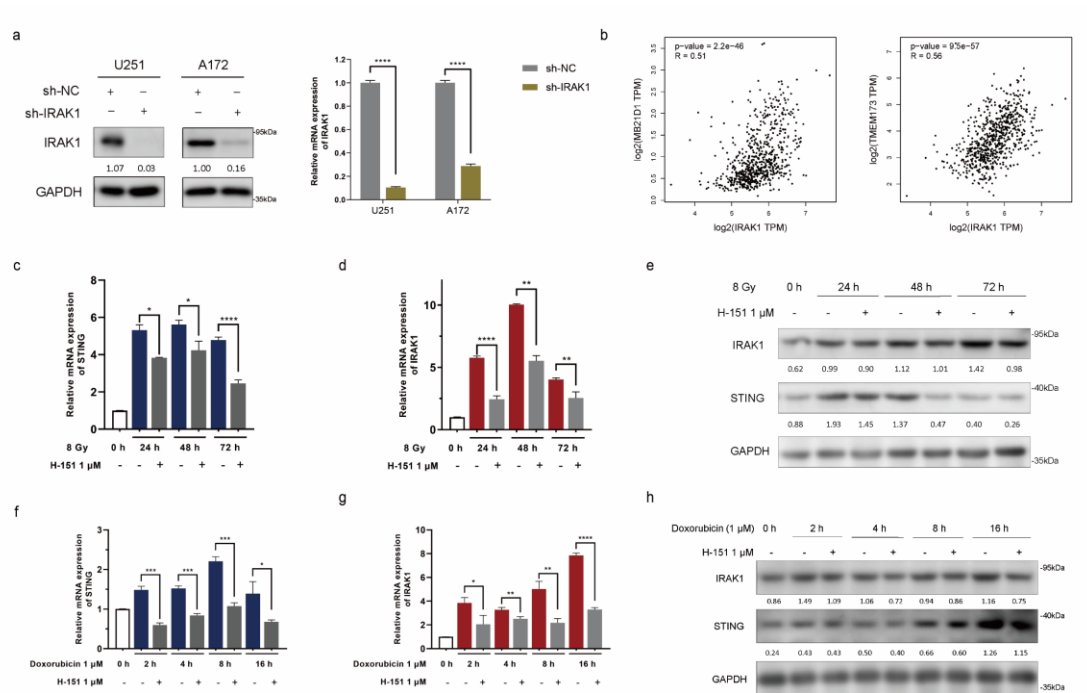


Supplementary information

Radiation induces IRAK1 expression to promote radioresistance by suppressing autophagic cell death via decreasing the ubiquitination of PRDX1 in glioma cells

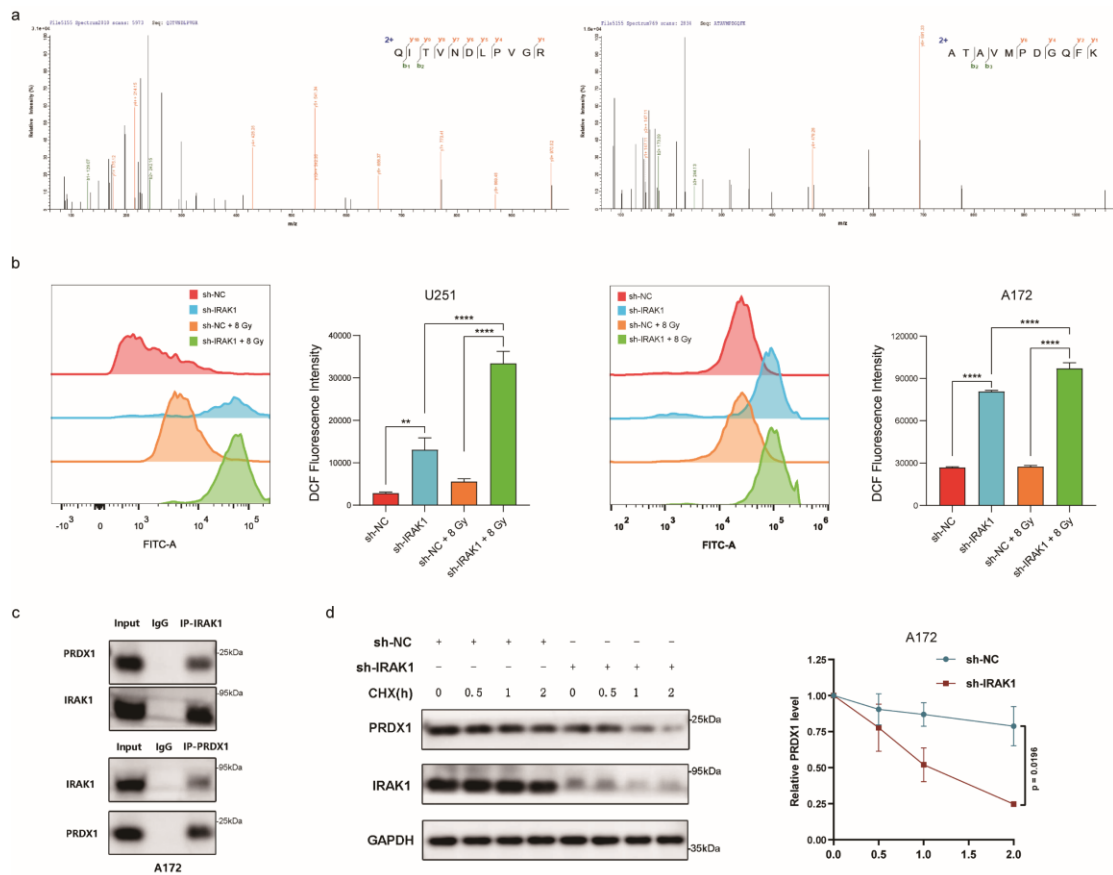
Jing Li et al.,



Supplementary Fig. S1

a Western blotting and qRT-PCR analysis of U251 and A172 cells transduced with lentiviral sh-NC or sh-IRAK1. **b** The Spearman correlation coefficients for IRAK1 expression with cGAS (MB21D1) and STING (TMEM173) based on GBM tumor and LGG tumor from TCGA datasets. Analyses were conducted using the GEPIA2 webserver (<http://gepia2.cancer-pku.cn/#index>). **c, d** Analysis of the mRNA expression of STING (**c**) and IRAK1 (**d**) in A172 cells treated with 8 Gy IR ± H-151 (1 μM) by qRT-PCR. **e** Western blotting analysis to assess the protein expression of IRAK1 and STING in A172 cells at 0, 24, 48, and 72 h after 8 Gy IR with or without H-151 (1 μM) treatment. **f, g**

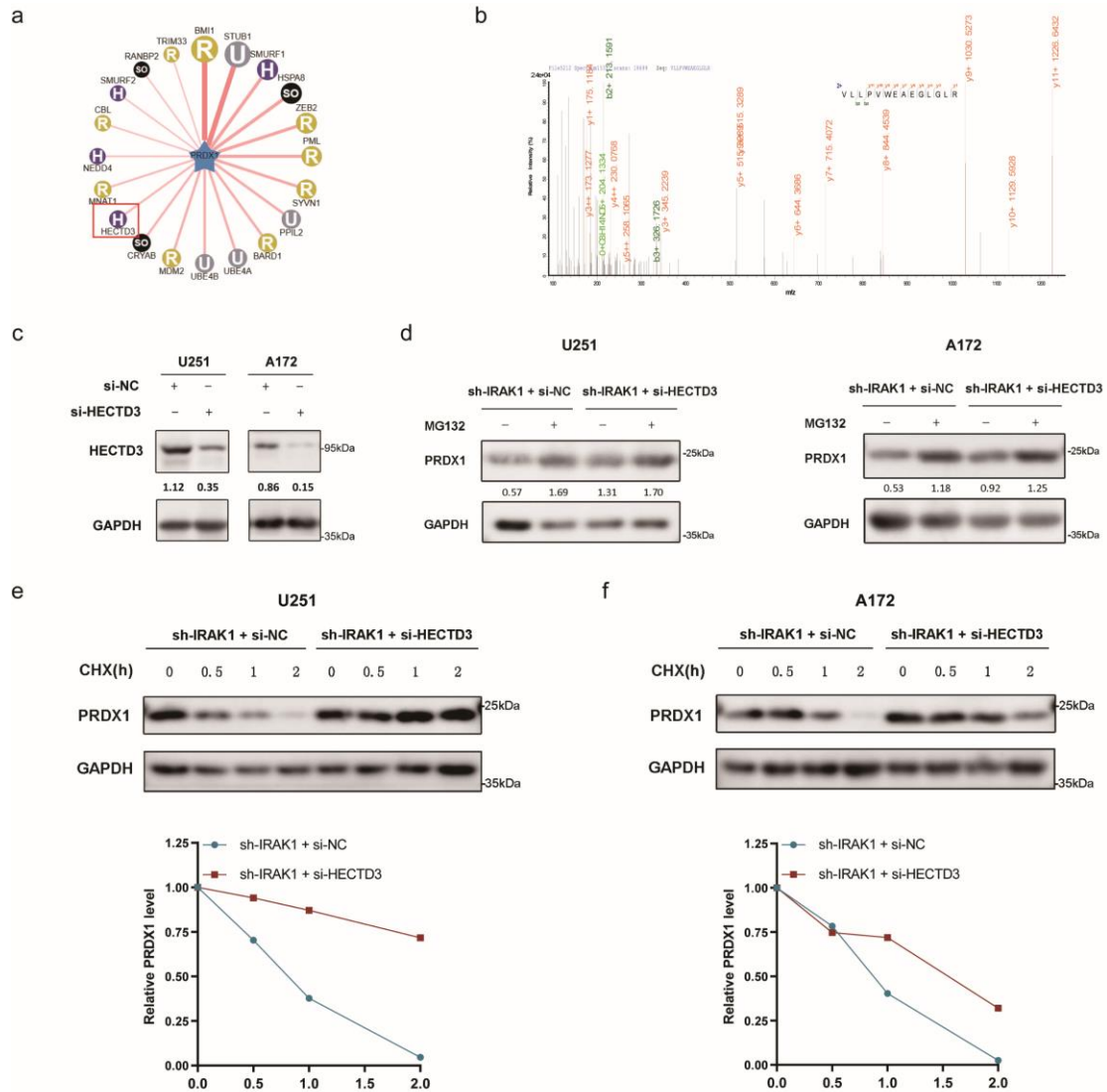
Analysis of the mRNA expression of STING (f) and IRAK1 (g) in A172 cells treated with doxorubicin (1 μ M) \pm H-151 (1 μ M) using qRT-PCR. h Effects of doxorubicin (1 μ M) \pm H-151 (1 μ M) treatment on IRAK1 and STING protein expression in A172 cells were evaluated by Western blotting. Data were presented as mean \pm SD from three independent experiments. * p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001.



Supplementary Fig. S2

a IRAK1 junction-specific peptides of PRDX1 were identified by IP combined with LC-MS/MS of anti-IRAK1 antibody in U251 cells. **b** Flow-cytometric analysis measured intracellular ROS levels in U251 and A172 cells after IRAK1 stably knockdown, followed by treatment with or without 8 Gy IR. **c** Co-IP detected the interaction of endogenous IRAK1 and PRDX1 in A172 cells. **d** The

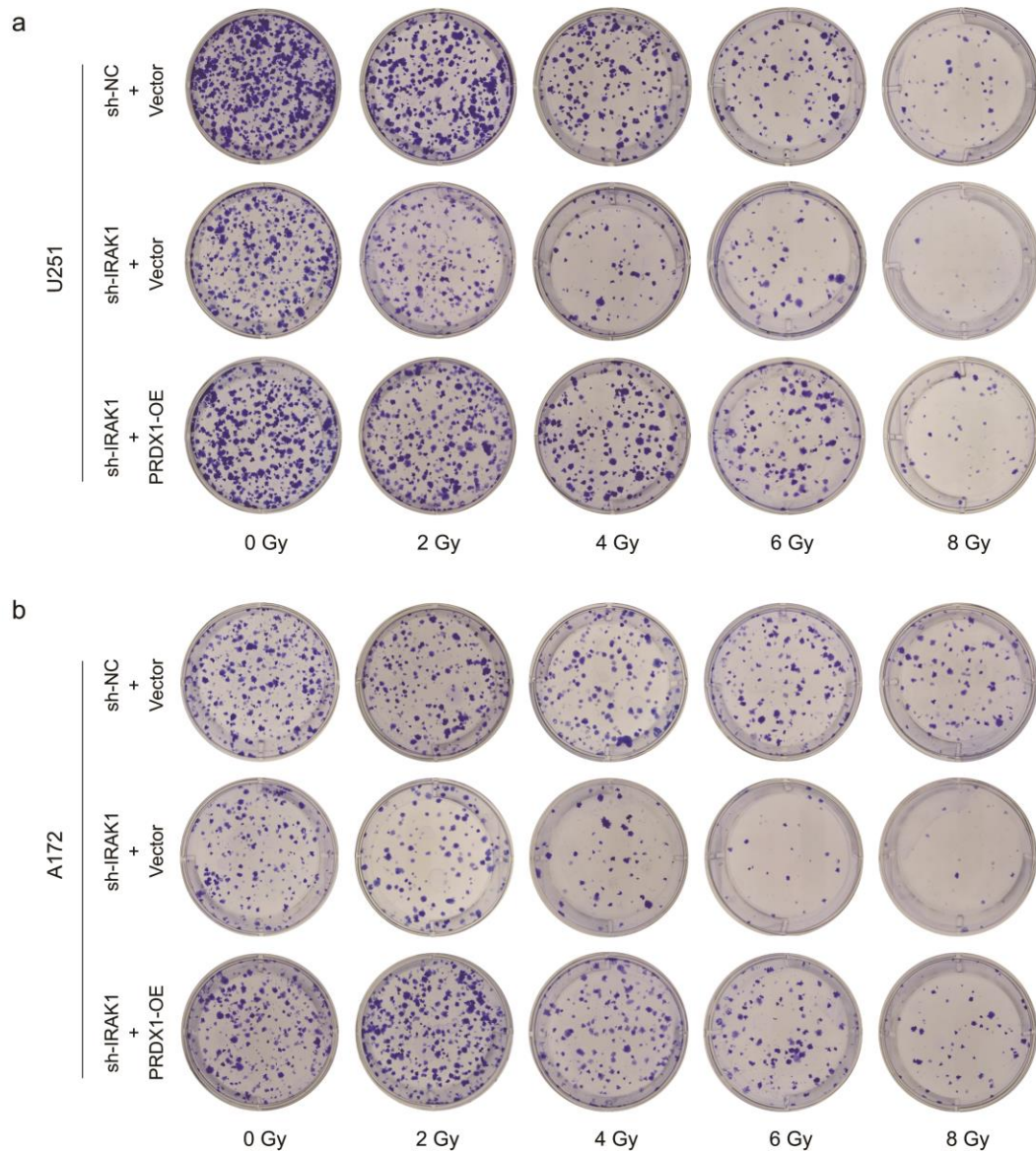
effect of CHX treatment (100 $\mu\text{g}/\text{mL}$) and greyscale quantification analysis upon IRAK1 knockdown in A172 cells.



Supplementary Fig. S3

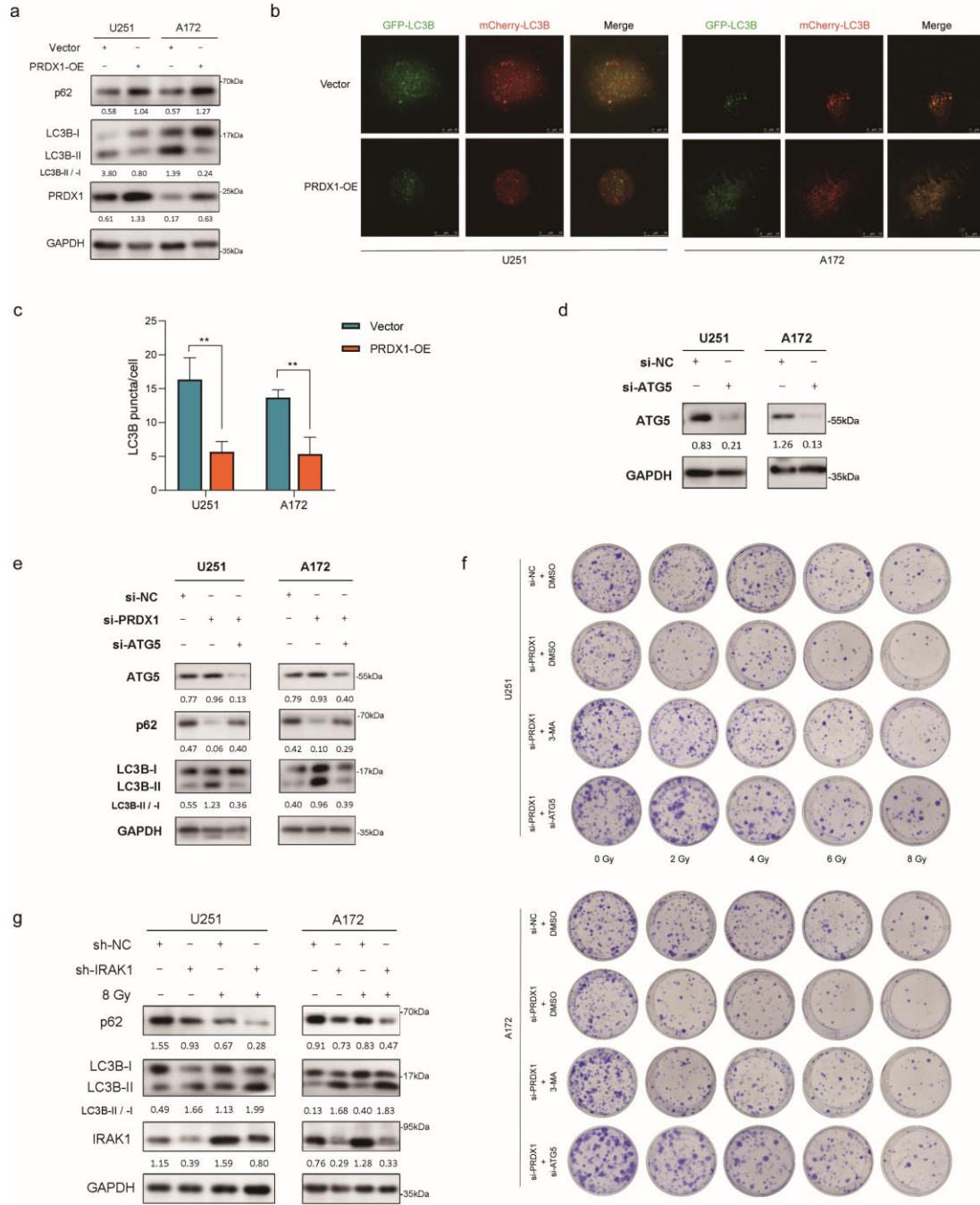
a Prediction of PRDX1 associated E3 ubiquitin ligase by using UbiBrowser webserver. Red rectangle indicated the interested one. **b** Peptides of E3 ligase HECTD3 were identified by IP combined with LC-MS/MS pulled down by anti-IRAK1 antibody in U251 cells. **c** Western blotting assays of U251 and A172 cells transiently transfected with si-NC or si-HECTD3 plasmids. **d** Western blotting detecting the effects of MG132 treatment on the protein levels of PRDX1 in sh-IRAK1 + si-NC and sh-IRAK1 + si-HECTD3 groups. **e, f** Western blotting assays were conducted

to analyze the stability of PRDX1 protein after being treated with CHX (100 $\mu\text{g}/\text{mL}$) for the different duration in sh-IRAK1 + si-NC and sh-IRAK1 + si-HECTD3 groups of glioma cells.



Supplementary Fig. S4

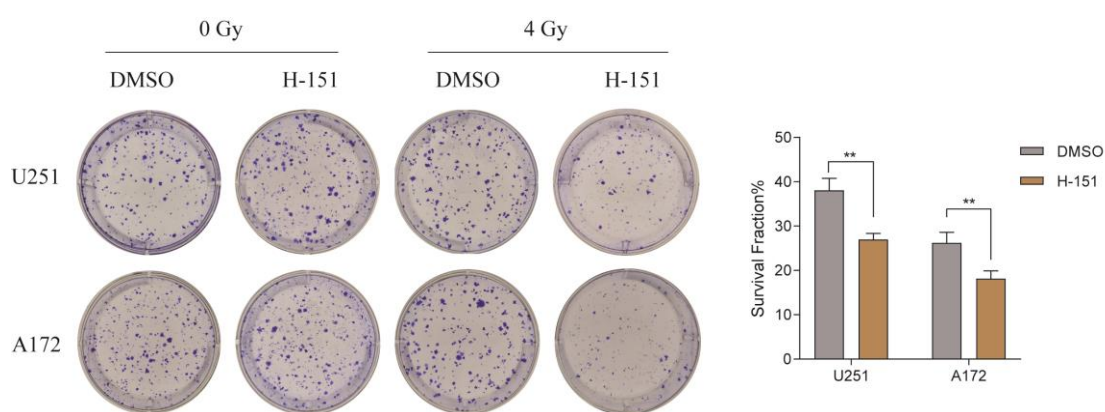
a, b Clone formation assays of sh-NC + Vector, sh-IRAK1 + Vector, and sh-IRAK1 + PRDX1 OE groups based on U251 (**a**) and A172 cells (**b**) following exposure to 0, 2, 4, 6, 8 Gy of X-rays.



Supplementary Fig. S5

a Western blotting detecting the protein levels of p62, LC3B, and PRDX1 in U251 and A172 cells with PRDX1 overexpression. **b, c** U251 and A172 cells with PRDX1 overexpression were transfected with mCherry-GFP-LC3B adenovirus for 48 h. Autophagic flux was measured by confocal microscopy (**b**), and the LC3B puncta was quantified (**c**). Scale bar, 10 μ m. Data were

presented as mean \pm SD from three repeats, ** $p < 0.01$. **d** The knockdown efficiency of si-ATG5 in U251 and A172 cells by Western blotting. **e** Western blotting assays detecting the changes of si-ATG5 on the protein levels of p62 and LC3B in PRDX1 silenced cells. **f** Representative images of clone formation assays of U251 and A172 cells transfected with si-PRDX1 with or without 3-MA (2.5 mM, 24 h) or si-ATG5 treatment before exposed to 0, 2, 4, 6, 8 Gy IR. **g** IRAK1 knockdown cells were accepted with 8 Gy IR. Western blotting assays were conducted to detect the protein levels of p62, LC3B, and IRAK1 in different groups. The LC3-II/LC3-I ratio was calculated based on densitometry analysis of both bands. The gray value ratios of other proteins/GAPDH were shown below each lane.



Supplementary Fig. S6

Representative images and the corresponding survival fraction histogram of clone formation assays treated with H-151 (1 μ M) or DMSO before being exposed to 4 Gy IR in U251 and A172 cells.

Supplementary Table S1. Radiobiological parameters of IRAK1 knockdown cells and control cells.

	D_0	D_q	N	SF_2
U251				
sh-NC	2.251 ± 0.121	1.311 ± 0.054	1.656 ± 0.171	0.568 ± 0.016

sh-IRAK1	1.360 ± 0.196 **	1.341 ± 0.154	2.925 ± 0.945	0.516 ± 0.017 *
A172				
sh-NC	1.998 ± 0.227	1.043 ± 0.133	1.454 ± 0.363	0.474 ± 0.037
sh-IRAK1	1.586 ± 0.026 *	0.795 ± 0.060 *	1.398 ± 0.096	0.372 ± 0.016 *

Supplementary Table S2. The sequences of lentivirus or plasmids used in this study.

Gene	Target sequences (5'-3')
shRNA sequences	
sh-NC	TTCTCCGAACGTGTCACGT
sh-IRAK1	GCCACCGCAGATTATCATCAA
siRNA sequences	
si-NC	UUCUCCGAACGUGUCACGUTT
si-PRDX1	ACUCAACUGCCAAGUGAUUTT
si-HECTD3	GCGGGAAGTGGGTTGAAT
si-ATG5	CCAUCAAUCCGAAACUCAUTT
si-FOXA2	GAACGGCATGAACACGTACAT

Supplementary Table S3. List of primers used in this study.

Primers' name	Sequence (5'-3')	
Primers for qRT-PCR		
IRAK1	Forward	TCAGCTTTGGGGTGGTAGTG
	Reverse	TAGATCTGCATGGCGATGGG
PRDX1	Forward	CTGCCAAGTGATTGGTGCTTCTG
	Reverse	AATGGTGCGCTTCGGGTCTGAT
cGAS	Forward	AAGGATAGCCGCCATGTTTCT
	Reverse	TGGCTTTCAGCAAAAGTTAGG
TMEM173/STING	Forward	AGCATTACAACAACCTGCTACG
	Reverse	GTTGGGGTCAGCCATACTCAG
GAPDH	Forward	GAAGAGAGAGACCCTCACGCTG
	Reverse	ACTGTGAGGAGGGGAGATTCAGT
Primers for CHIP-qPCR		
IRAK1-P1	Forward	AAACCTGTCTGAATGTACCTGT
	Reverse	TGCTGCCCATCTTTTCCAAT
IRAK1-P2	Forward	TGGCTCCTCCTGTGTCTCAT
	Reverse	TTGCCTGGTCAGAGAACCAC
IRAK1-P3	Forward	GAGTAAGTCTGCGTTGCTGC
	Reverse	CCTGGGCATCGTCCTTGATT

Supplementary Table S4. List of antibodies used in the study.

Antibody	Company	Catalog no.	Dilution
Western blot			
GAPDH antibody, Rabbit Polyclonal	Proteintech	10494-1-AP	1:10000
IRAK1 antibody, Rabbit Polyclonal	Proteintech	10478-2-AP	1:1000
IRAK1 antibody, Rabbit Monoclonal	CST	4504S	1:1000
E-cadherin antibody, Rabbit Polyclonal	Proteintech	20874-1-AP	1:5000
N-cadherin antibody, Rabbit Polyclonal	Proteintech	22018-1-AP	1:2000
Vimentin antibody, Rabbit Polyclonal	Proteintech	10366-1-AP	1:1000
TMEM173/STING antibody, Rabbit Polyclonal	Proteintech	19851-1-AP	1:1000
PRDX1 antibody, Rabbit Polyclonal	Proteintech	15816-1-AP	1:1000
GST Tag antibody, Rabbit Polyclonal	Proteintech	10000-0-AP	1:1000
Ubiquitin antibody, Rabbit Polyclonal	Proteintech	10201-2-AP	1:500
HECTD3 antibody, Rabbit Polyclonal	Proteintech	11487-1-AP	1:2000
LC3B antibody, Rabbit Polyclonal	Proteintech	18725-1-AP	1:1000
Anti-SQSTM1/p62 antibody, Rabbit Monoclonal	Abcam	ab109012	1:2000
AKT antibody, Rabbit Polyclonal	Proteintech	10176-2-AP	1:1000
Phospho-AKT (Ser473) antibody, Rabbit Polyclonal	Proteintech	28731-1-AP	1:1000
Phospho-AKT (Thr308) antibody, Rabbit Polyclonal	Proteintech	29163-1-AP	1:2000
mTOR (7C10) antibody, Rabbit Monoclonal	CST	2983S	1:1000
Phospho-mTOR (Ser2448) (D9C2) XP® antibody, Rabbit Monoclonal	CST	5536S	1:1000
DYKDDDDK (FLAG) Tag antibody, Rabbit Polyclonal	Proteintech	20543-1-AP	1:2000
HRP-labeled Goat Anti-Rabbit IgG (H+L) antibody	Beyotime	A0208	1:2000
HRP-labeled Goat Anti-Mouse IgG (H+L) antibody	Beyotime	A0216	1:1000
Anti-FOXA2 antibody-ChIP Grade, Rabbit Monoclonal	Abcam	ab108396	1:500
ATG5 antibody, Rabbit Polyclonal	Proteintech	10181-2-AP	1:2000
Immunofluorescence			
IRAK1 antibody, Mouse Monoclonal	Proteintech	66653-1-Ig	1:100
PRDX1 antibody, Rabbit Polyclonal	Proteintech	15816-1-AP	1:150
IRAK1 antibody, Rabbit Polyclonal	Proteintech	10478-2-AP	1:200
cGAS antibody, Rabbit Polyclonal	Proteintech	26416-1-AP	1:100

TMEM173/STING antibody, Rabbit Polyclonal	Proteintech	19851-1-AP	1:200
Phospho-Histone H2A.X (Ser139) (20E3) antibody, Rabbit mAb	CST	9718S	1:400
Goat Anti-Rabbit IgG, Cy3 Conjugated secondary antibody	zhuangzhibio	EK022	1:100
Goat Anti-Mouse IgG, Cy3 Conjugated secondary antibody	zhuangzhibio	EK012	1:100
Goat Anti-Rabbit IgG, FITC Conjugated secondary antibody	zhuangzhibio	EK023	1:100

Co-Immunoprecipitation

Normal Rabbit IgG	CST	2729S	1:100
IRAK1 antibody, Rabbit Monoclonal	CST	4504S	5 µg
PRDX1 antibody, Rabbit Polyclonal	Proteintech	15816-1-AP	5 µg
HECTD3 antibody, Rabbit Polyclonal	Proteintech	11487-1-AP	5 µg
MYC Tag antibody, Rabbit Polyclonal	Proteintech	16286-1-AP	3 µg
Anti-FLAG® M2 antibody produced in mouse	Sigma	F1804	3 µg

Chromatin Immunoprecipitation (ChIP)

Anti-FOXA2 antibody-ChIP Grade, Rabbit Monoclonal	Abcam	ab108396	1.5 µg
Normal Rabbit IgG	CST	2729S	1.5 µg

Immunohistochemistry

IRAK1 antibody, Rabbit Polyclonal	Proteintech	10478-2-AP	1:600
PRDX1 antibody, Rabbit Polyclonal	Proteintech	15816-1-AP	1:200
LC3B antibody, Rabbit Polyclonal	Proteintech	18725-1-AP	1:400
SQSTM1/p62 antibody, Rabbit Polyclonal	Proteintech	18420-1-AP	1:100
Ki67 antibody, Rabbit Polyclonal	Proteintech	27309-1-AP	1:10000
