Supplementary materials

Supplementary Table S1 Information of the antibodies used in the present work

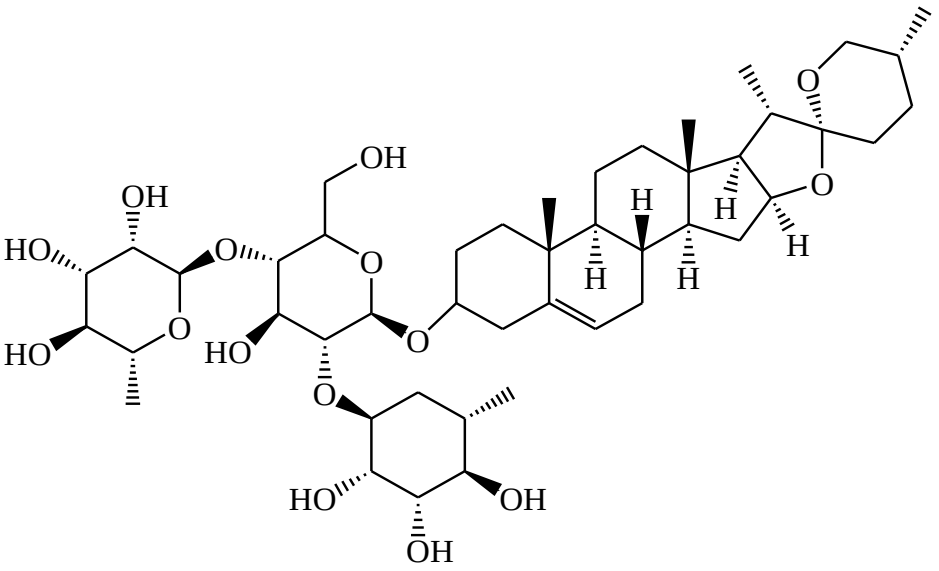
Primary antibody	Source	Dilution	Company
TIGAR	Rabbit	1: 500	Bioworld Technology, USA
p53	Rabbit	1: 1000	Proteintech Group, Chicago, USA
Bax	Rabbit	1: 1000	Proteintech Group, Chicago, USA
Bcl-2	Rabbit	1: 500	Proteintech Group, Chicago, USA
Cleaved-Caspase 3	Rabbit	1: 500	Proteintech Group, Chicago, USA
Cleaved-Caspase 9	Rabbit	1: 1000	Proteintech Group, Chicago, USA
Cleaved-PARP	Rabbit	1:1000	Proteintech Group, Chicago, USA
AKT	Rabbit	1: 1000	Proteintech Group, Chicago, USA
p-AKT	Rabbit	1: 500	Bioworld Technology, USA
m-TOR	Rabbit	1: 1000	Proteintech Group, Chicago, USA
p-mTOR	Rabbit	1: 200	Bioworld Technology, USA
LC3	Rabbit	1: 2000	Proteintech Group, Chicago, USA
Beclin-1	Rabbit	1: 1000	Proteintech Group, Chicago, USA
CDK-5	Rabbit	1: 1000	Proteintech Group, Chicago, USA
ATM	Rabbit	1: 200	Bioworld Technology, USA
p-ATM	Rabbit	1: 100	Bioworld Technology, USA
GAPDH	Rabbit	1: 1000	Proteintech Group, Chicago, USA

Supplementary Table S2 The primer sequences used for real-time PCR assay in the present work.

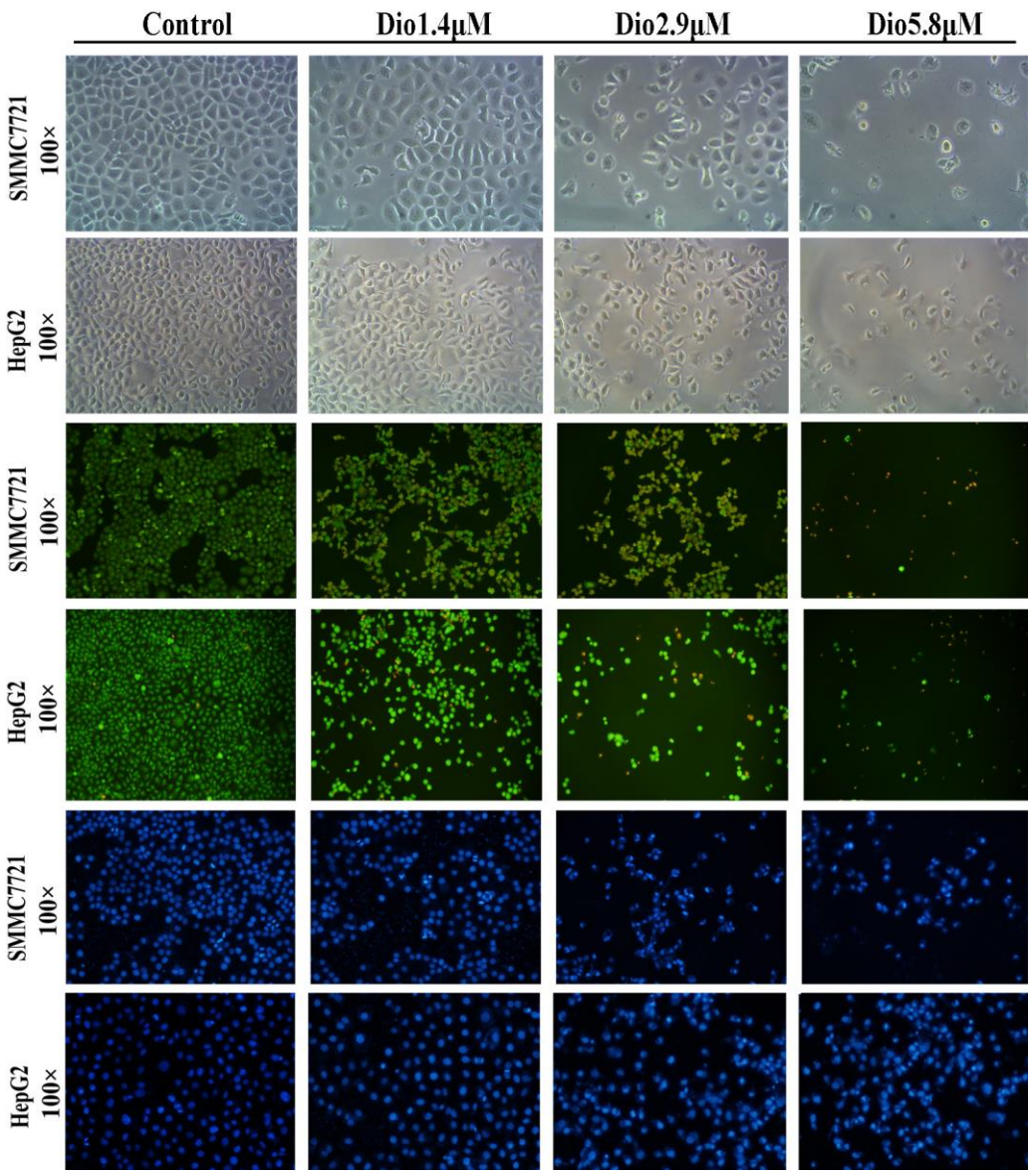
Gene	Forward primer(5’-3’)	Reverse primer(5’-3’)
TIGAR Rats	GCTTCAGACGTATATATAGA	GGGGCTATTCTTGGTAGTAA
TIGAR Human	GGCTTCGGGAAAGGAAATA	AACCTGGAATAC CGCTGTCT
TIGAR mice	CGGCAGGTCTTAGATAGCTT	GGCAGCCGGCATCAAAAACA

Supplementary Table S3 IC_{50} (μM) values of dioscin against cancer cells

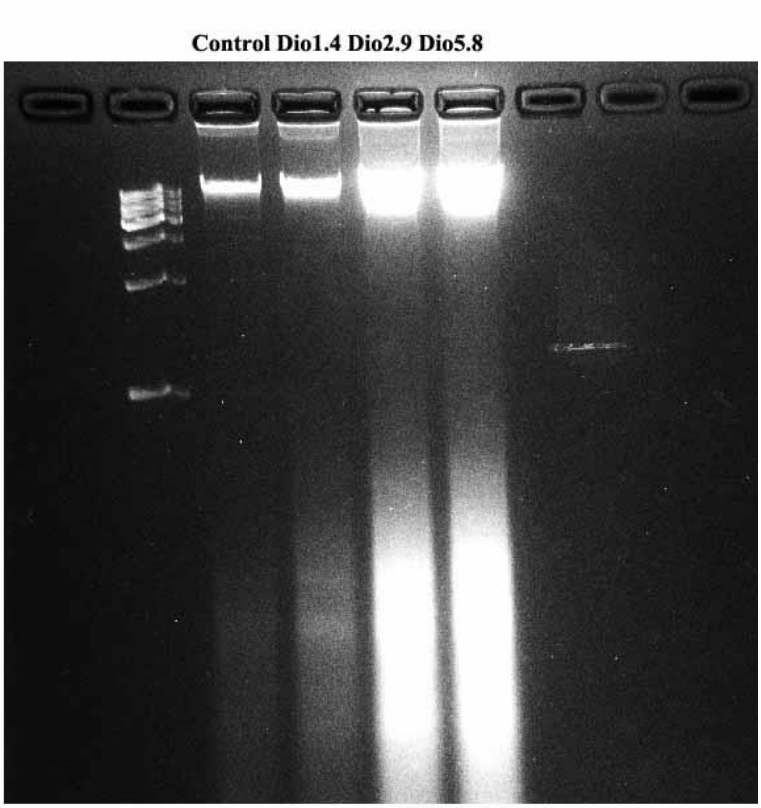
Time	HepG2	SMMC7721	Huh-7	BEL-7402
12 h	3.91	2.92	6.32	8.98
24 h	3.03	2.55	4.17	6.72
36 h	2.33	2.21	3.76	5.85



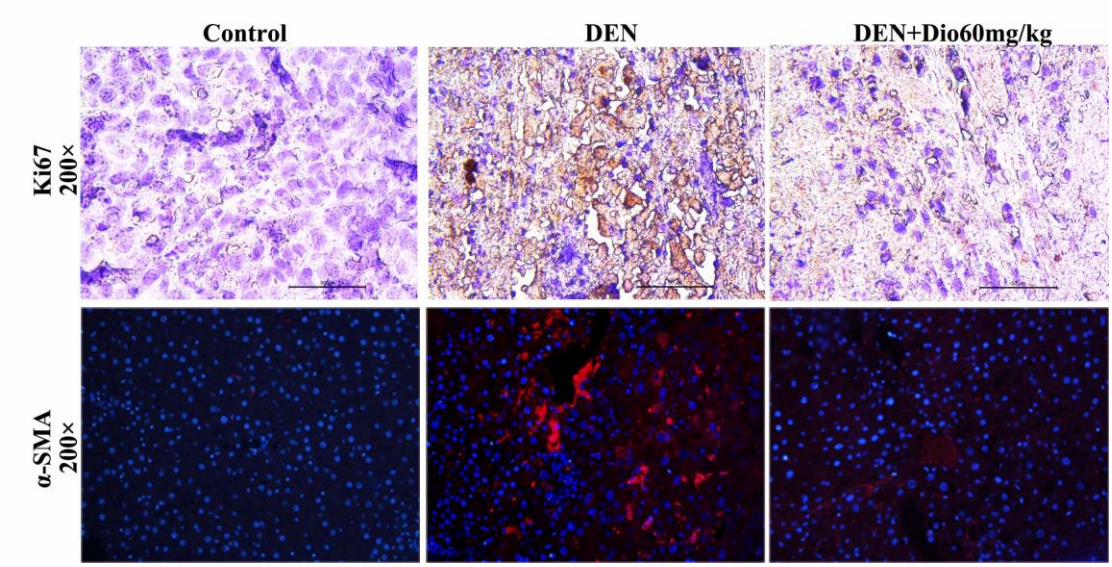
Supplementary Figure S1 Chemical structure of dioscin



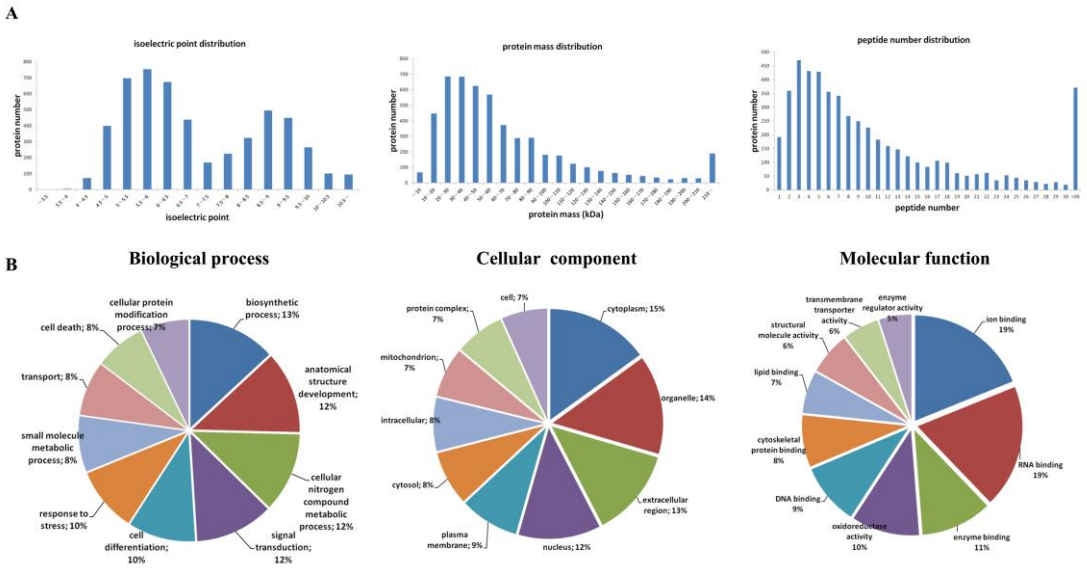
Supplementary Figure S2 Effects of dioscin on structures and fluorescence images by AO/EB and DAPI staining in SMMC7721 and HepG2 cells.



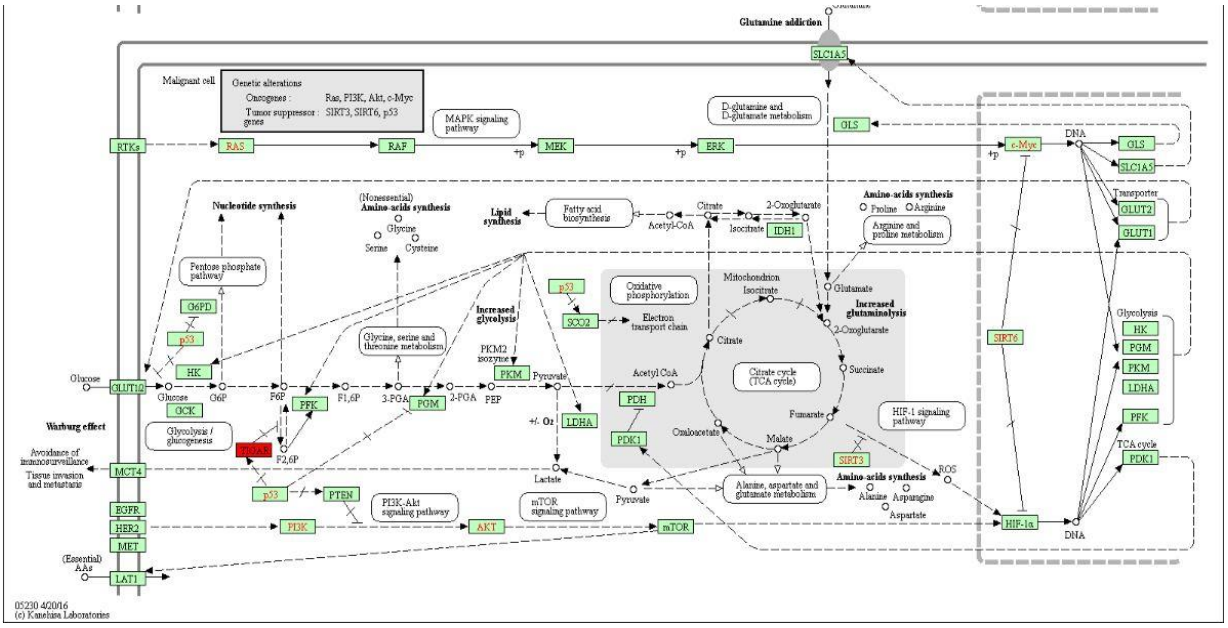
Supplementary Figure S3 Effects of dioscin on apoptotic cell death in SMMC7721 cells. Fragmentation of DNA was isolated using Agarose gel electrophoresis.



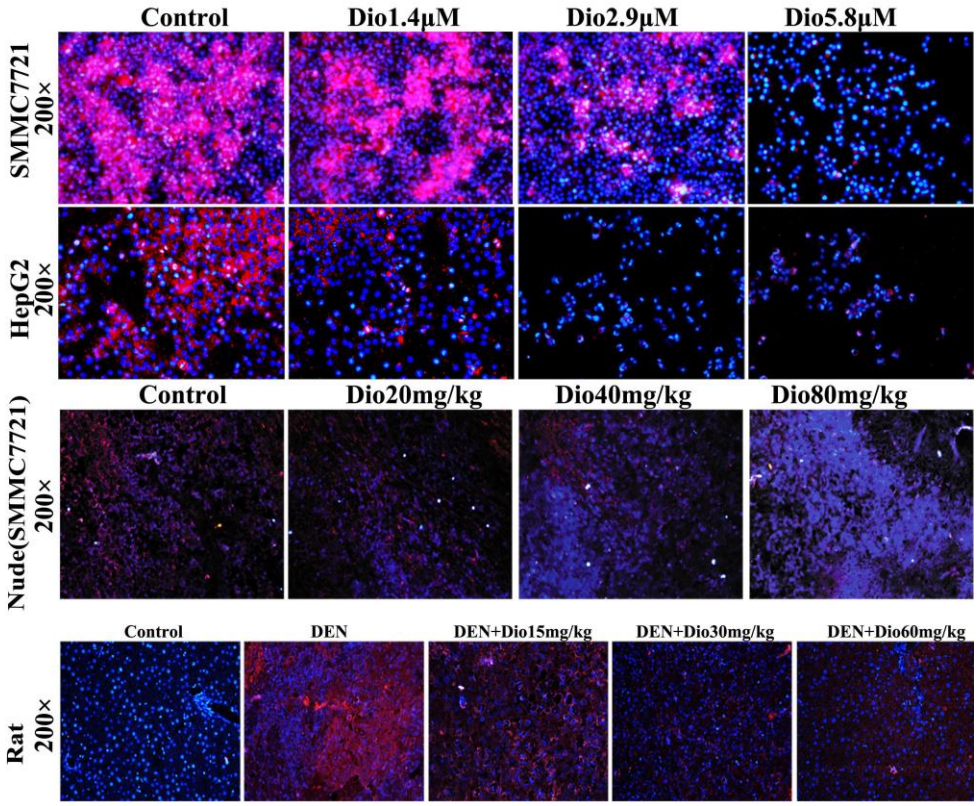
Supplementary Figure S4 Effects of dioscin on the expression levels of Ki67 and α-SMA in rats.



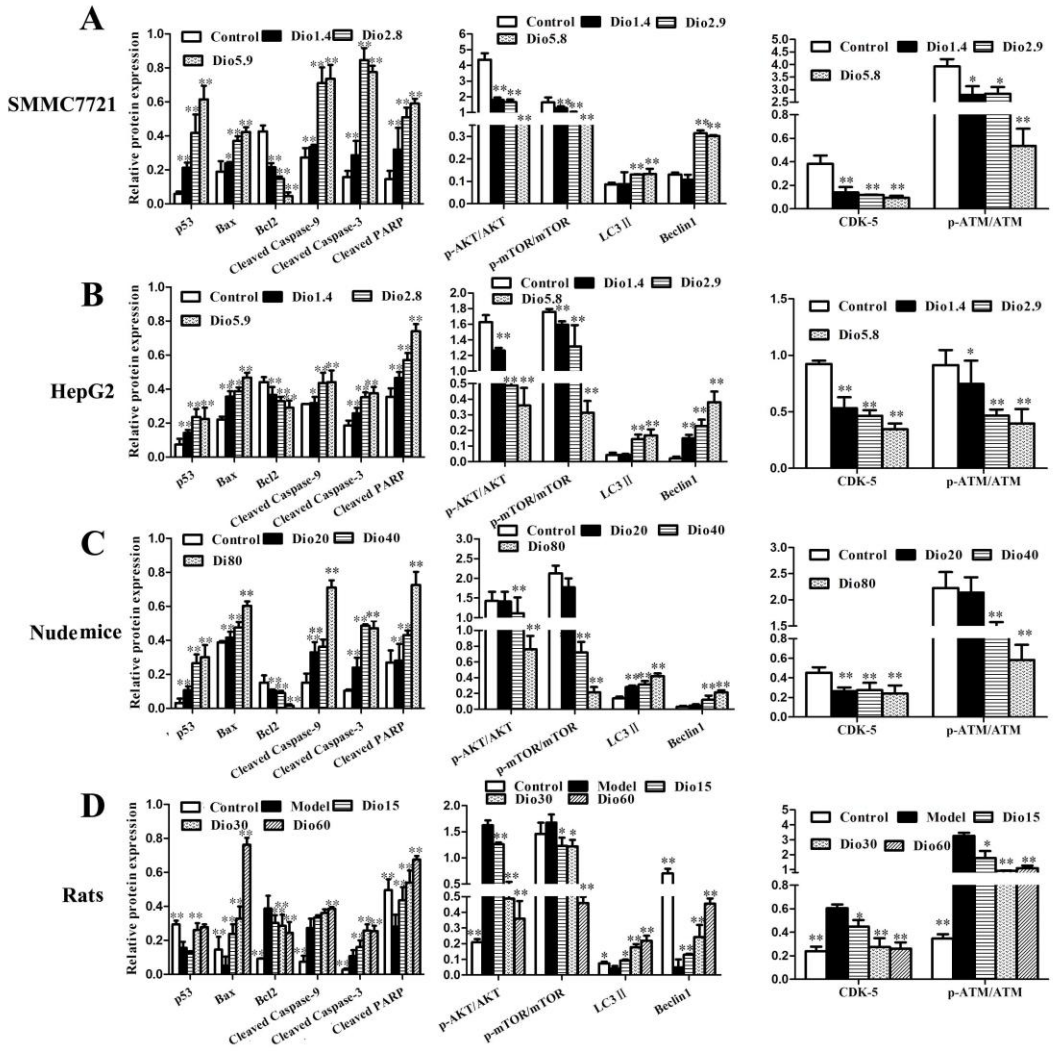
Supplementary Figure S5 Information and classification of the differentially expressed proteins in SMMC7721 cells caused by dioscin. (A) The protein mass distribution, peptide length distribution and isoelectric point distribution of the differentially expressed proteins in SMMC7721 cells caused by dioscin. (B) Gene Classification of the differentially expressed proteins identified by proteomics according to the cellular component, molecular function and biological process by Gene Ontology (GO) analysis.



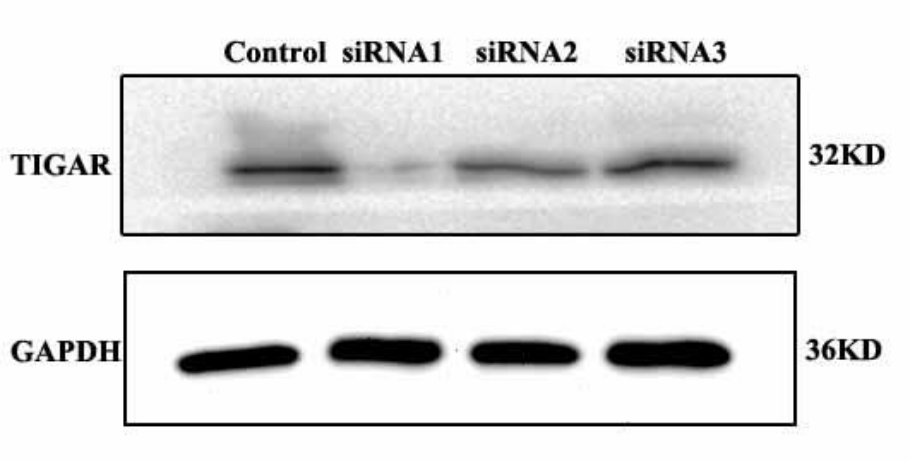
Supplementary Figure S6 Pathway analysis using the KEGG database. The map is the central carbon metabolism in cancer pathway and the red proteins in the pathway are identified by iTRAQ-based proteomics from SMMC7721 cells treated with and without dioscin.



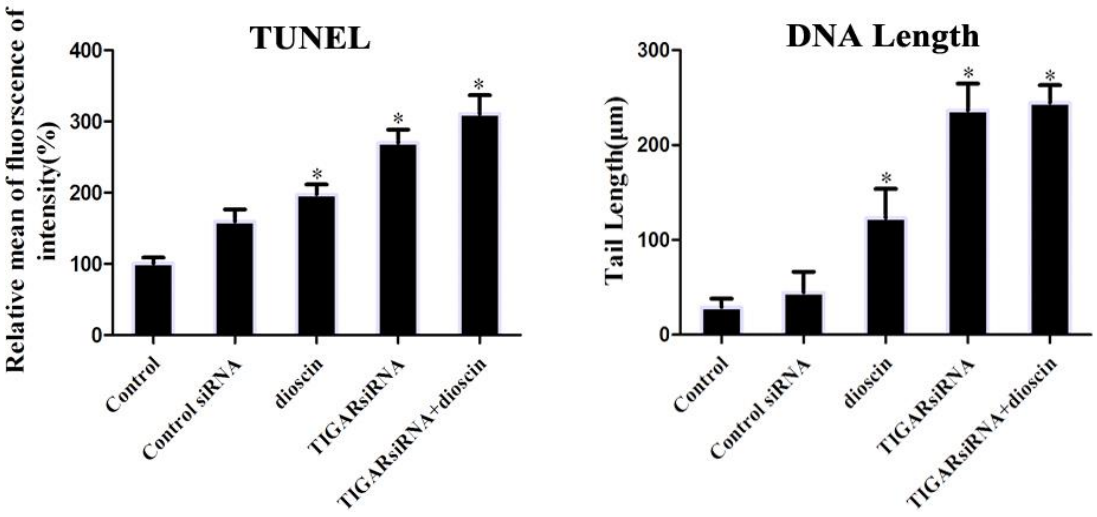
Supplementary Figure S7 Effects of dioscin on the expression levels of TIGAR based on immunofluorescence assay.



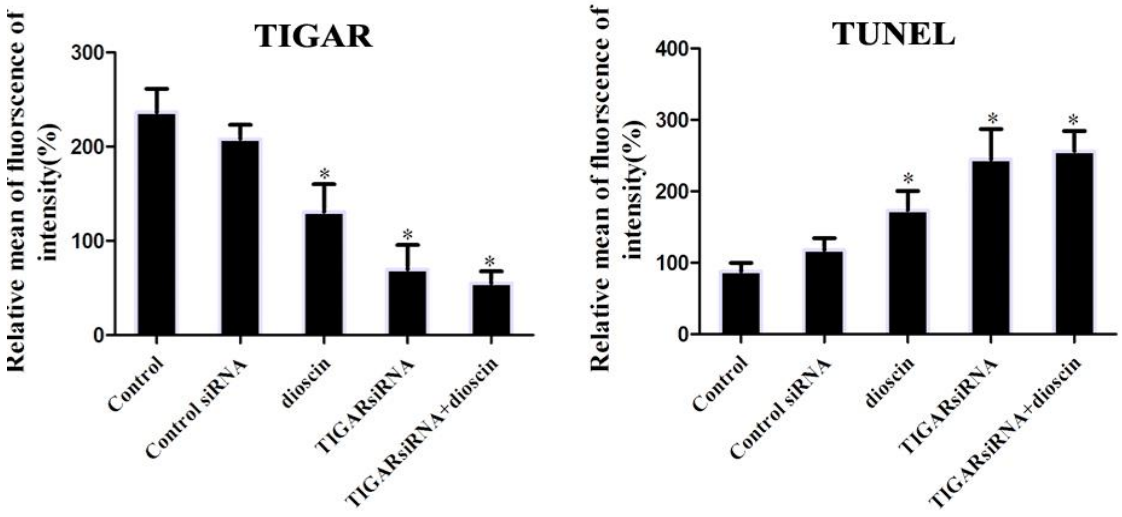
Supplementary Figure S8 Effects of disocin on the expression levels of the proteins associated with TIGAR-mediated signal pathway *in vitro* and *in vivo*. (A-C).Statistic analysis of the expression levels of the proteins including p53, Bax, Bcl-2, Cleaved PARP Cleaved Caspase-3/9, AKT, p-AKT, mTOR, p-mTOR, LC3, Beclin1, CDK5, ATM, p-ATM in cells and in nude mice. Data are presented as mean ± SD (n = 5). $^{*}p < 0.05$ versus control group. (D). the expression levels of the proteins including p53, Bax, Bcl-2, Cleaved PARP, Cleaved Caspase-3/9, AKT, p-AKT, mTOR, p-mTOR, LC3, Beclin1, CDK5, ATM, p-ATM in Rats. Data are presented as mean ± SD (n = 5). $^{*}p < 0.05$ versus model group.



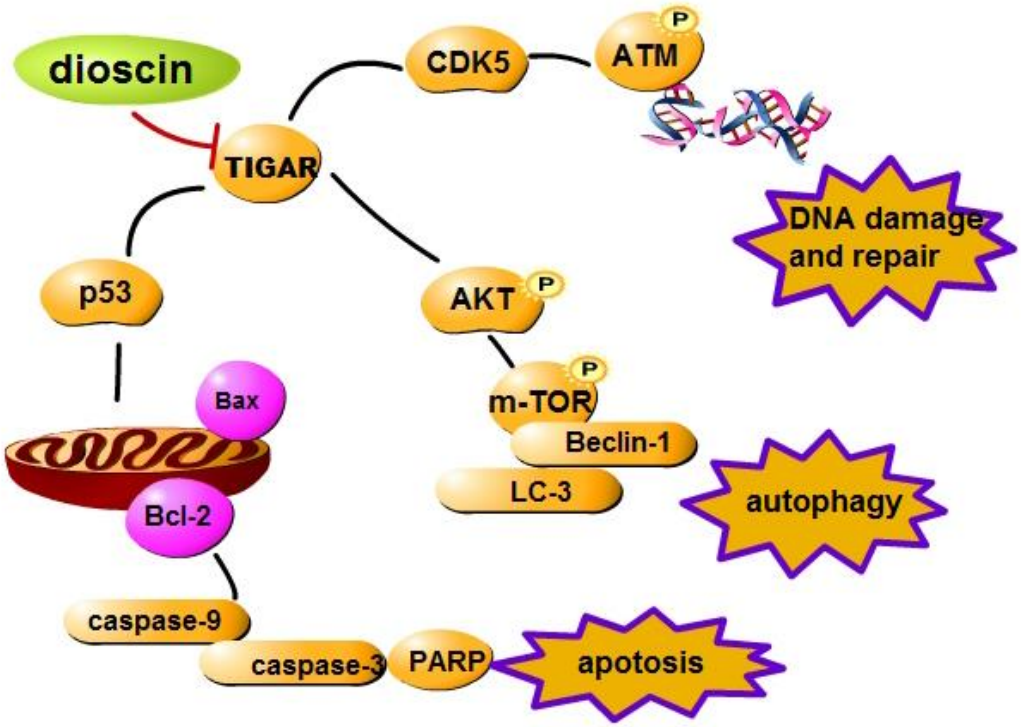
Supplementary Figure S9 The expression levels of the TIGAR using different siRNAs.



Supplementary Figure S10 Effects of diosicn on cell apoptosis and DNA damage on SMMC7721 cells after TIGAR-siRNA transfection based on TUNEL and commet assays. All data are expressed as mean ± SD (n = 5). *$p < 0.05$ versus control group.



Supplementary Figure S11 Effects of diosicn on the expression level of TIGAR and cell apoptosis on SMMC7721 cells after TIGAR-siRNA transfection based on immuno-fluorescence and TUNEL assays. All data are expressed as mean ± SD (n = 5). *p < 0.05 versus control group.



Supplementary Figure S12 Schematic diagram of the mechanisms of dioscin against HCC. Dioscin decreased the expression level of TIGAR by regulating p53, AKT/mTOR, CDK5/ATM to cause apoptosis, autophagy and DNA damage.