Poent effects of dioscin against pancreatic cancer via miR-149-3P-mediated inhibition of AKT1 signaling pathway

Lingling Si, Lina Xu, Lianhong Yin, Yan Qi, Xu Han, Youwei Xu, Yanyan Zhao, Changyuan Wang, Kexin Liu, Jinyong Peng*

College of Pharmacy, Dalian Medical University, Western 9 Lvshunnan Road, Dalian 116044, China

Corresponding author,
Dr. Jinyong Peng
College of Pharmacy
Dalian Medical University
Dalian, China
Tel.: +86 411 8611 0411
Fax: +86 411 8611 0411
Email: jinyongpeng2005@163.com

Supplemental Table 1. The primer sequences used for real-time PCR assay in the present work.

Gene	Accession number	Primer (5'–3')
MiR-149-3P	MIMAT0004609	AGGGAGGGACGGGGGCT

Supplemental Table 2. The information of the antibodies used in the present work.

Antibody	Source	Dilutions	Company
AKT1	Rabbit	1:1000	Proteintech Group, Chicago, USA
Bax	Rabbit	1:1000	Proteintech Group, Chicago, USA
Bcl-2	Rabbit	1:1000	Proteintech Group, Chicago, USA
Apaf-1	Rabbit	1:1000	Proteintech Group, Chicago, USA
Cleaved caspase-3	Rabbit	1:1000	Proteintech Group, Chicago, USA
Cytochrome C	Rabbit	1:1000	Proteintech Group, Chicago, USA
Cleaved caspase-9	Rabbit	1:1000	Invitrogen, CA, USA
Cleaved PARP	Rabbit	1:1000	Abcam, Cambridge, UK

Supplemental Table 3. The information of the sequences used in the present work.

Gene	Primer (5'–3')
MiR-149-3P inhibitor	GCACAGCCCCCGUCCCUCCCU
MiR-149-3P mimics	Forward: AGGGAGGGACGGGGGCUGUGC ; Reverse: ACAGCCCCCGUCCCUCCCUUU
AKT1 siRNA	Forward: UGCCCUUCUACAACCAGGATT; Reverse: UCCUGGUUGUAGAAGGGCATT

Supplemental Table 4. Differentially expressed microRNAs caused by dioscin in ASPC-1 cells using microRNA microarray analysis.

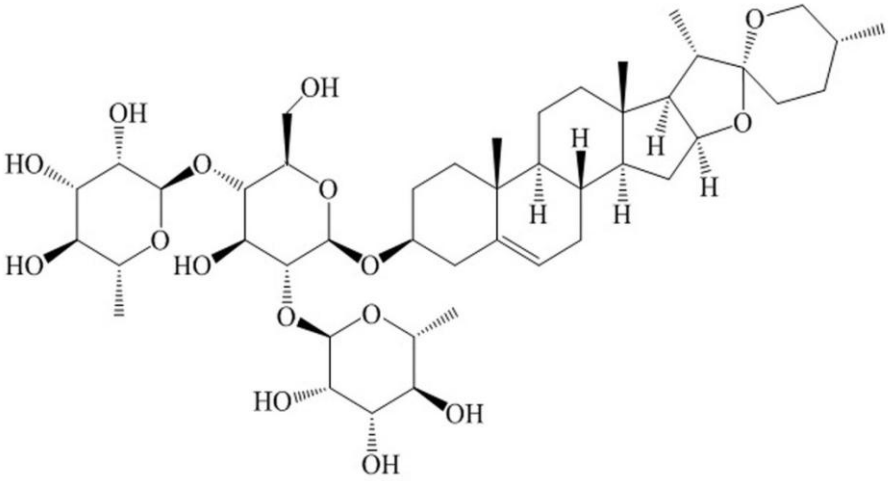
ID	Gene name	Fold change [a]	P-value [b]
169087	hsa-miR-149-3p	12.495	0.042
42832	hsa-miR-638	9.875	0.047
42466	ebv-miR-BART18-3p	7.840	0.015
169009	hsa-miR-548ap-5p/hs	7.197	0.035
148085	hsa-miR-3687	6.480	0.010
148652	hsa-miR-620	6.407	0.013
148684	hsa-miR-628-3p	5.923	0.006
147916	hsa-miR-3126-3p	5.767	0.010
169189	hsa-miR-4795-5p	5.650	0.002
147706	hsa-miR-4255	5.369	0.024
169239	hsa-miR-4732-5p	5.139	0.007
147926	hsa-miR-4329	4.642	0.024
169228	hsa-miR-4698	4.551	0.009
169271	hsa-miR-4784	4.330	0.040
147614	hsa-miR-4299	4.286	0.007
168639	hsa-miR-4533	4.111	0.006
17358	ebv-miR-BART16	4.010	0.037
148263	hsa-miR-1273e	3.927	0.039
168597	hsa-miR-5699-3p	3.916	0.049
168911	hsa-miR-4682	3.744	0.011
147584	hsa-miR-548t-5p	3.593	0.008
146117	hsv1-miR-H6-3p	3.572	0.045
169395	hsa-miR-4484	3.570	0.040
13137	hsa-miR-519a-5p	3.520	0.022
42490	hsa-miR-505-5p	3.395	0.012
146072	hsa-miR-1469	3.328	0.028
27537	ebv-miR-BART13-3p	3.300	0.022
168637	hsa-miR-3940-5p	3.261	0.032
168978	hsa-miR-371b-5p	3.226	0.006
169050	hsa-miR-4787-5p	3.222	0.027
148068	hsa-miR-758-5p	3.199	0.020
168994	hsa-miR-3591-5p	3.166	0.001
42442	hsa-miR-498	3.126	0.004
168863	hsa-miR-4441	3.079	0.015
169185	hsa-miR-5187-3p	2.886	0.011
168640	hsa-miR-4475	2.884	0.001
169341	hsa-miR-4632-3p	2.745	0.002
17327	hsa-miR-630	2.737	0.038
146158	hsa-miR-3202	2.719	0.043
168557	hsa-miR-4777-5p	2.666	0.006
42522	ebv-miR-BART19-3p	2.632	0.042

168899	hsa-miR-1285-5p	2.621	0.001
17492	sv40-miR-S1-5p	2.597	0.016
168852	hsa-miR-4764-5p	2.566	0.024
169035	hsa-miR-4658	2.534	0.043
46479	hsa-miR-1304-5p	2.482	0.016
146068	hsa-miR-1915-3p	2.406	0.004
168805	hsa-miR-4478	2.349	0.029
168963	hsa-miR-664b-5p	2.302	0.011
168661	hsa-miR-4531	2.289	0.018
146165	hsa-miR-1973	2.242	0.016
147836	hsv2-miR-H7-5p	2.236	0.004
168605	hsa-miR-4653-3p	2.236	0.003
168653	hsa-miR-3158-5p	2.213	0.009
148420	hsa-miR-3607-3p	2.208	0.000
168709	hsa-miR-4429	2.178	0.027
42696	hsa-miR-943	2.136	0.009
169031	hsa-miR-4726-5p	2.129	0.025
169254	hsa-miR-4762-3p	0.043	0.048
42672	hsa-miR-323b-5p	0.049	0.004
148674	hsa-miR-4321	0.065	0.026
168915	hsa-miR-4780	0.083	0.039
168871	hsa-miR-151a-5p	0.119	0.005
10985	hsa-miR-191-5p	0.126	0.032
169214	hsa-miR-4638-5p	0.132	0.024
42887	hsa-miR-331-3p	0.172	0.002
11260	hsa-miR-151a-5p	0.173	0.001
145638	hsa-miR-29a-5p	0.183	0.001
27533	hsa-miR-320a	0.185	0.011
42923	hsa-miR-30c-5p	0.192	0.007
11020	hsa-miR-22-3p	0.198	0.008
169129	hsa-miR-4284	0.200	0.010
148430	hsa-miR-374c-5p	0.201	0.010
169407	hsa-miR-4301	0.202	0.005
146159	hsv1-miR-H4-3p	0.209	0.009
17280	hsa-miR-15b-5p	0.210	0.031
42783	hsa-miR-197-3p	0.218	0.008
145634	hsa-miR-132-5p	0.219	0.009
145701	hsa-miR-668-3p	0.227	0.022
168798	hsa-miR-4668-5p	0.228	0.045
14301	hsa-miR-361-5p	0.243	0.005
169408	hsa-miR-181d-5p	0.249	0.001
148098	hsa-miR-374b-5p	0.250	0.025
13147	hsa-miR-96-5p	0.273	0.002
19582	hsa-miR-106b-5p	0.276	0.028

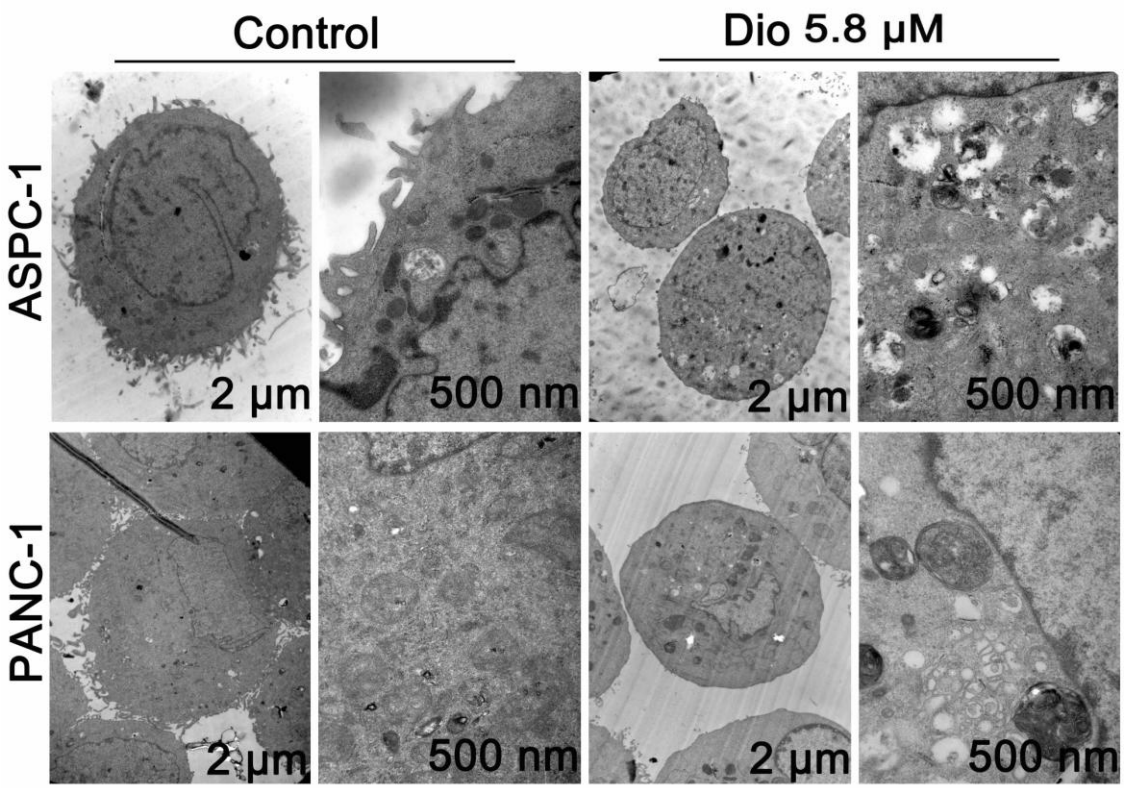
11023	hsa-miR-222-3p	0.278	0.002
145676	hsa-miR-30e-3p	0.284	0.011
4610	hsa-miR-126-3p	0.300	0.011
147588	hsa-miR-4288	0.324	0.000
10975	hsa-miR-182-5p	0.326	0.007
42446	hsa-miR-576-5p	0.328	0.005
42744	hsa-miR-23a-3p	0.328	0.008
146112	hsa-miR-30b-5p	0.344	0.040
145845	hsa-miR-20a-5p	0.354	0.037
46438	hsa-let-7g-5p	0.362	0.007
169070	hsa-miR-4695-3p	0.385	0.003
46732	hsa-miR-1264	0.395	0.007
28966	hsa-miR-574-3p	0.399	0.027
147776	hsa-miR-4317	0.401	0.015
169336	hsa-miR-17-5p	0.406	0.030
9938	hsa-let-7i-5p	0.444	0.036
42730	hsa-miR-423-3p	0.463	0.030
17506	hsa-miR-24-3p	0.477	0.033
145742	hsa-miR-935	0.479	0.022
147199	hsa-miR-27b-3p	0.480	0.039
147506	hsa-miR-21-5p	0.491	0.029

[a] The ratio of normalized intensities between two conditions (use normalized data, ratio scale).

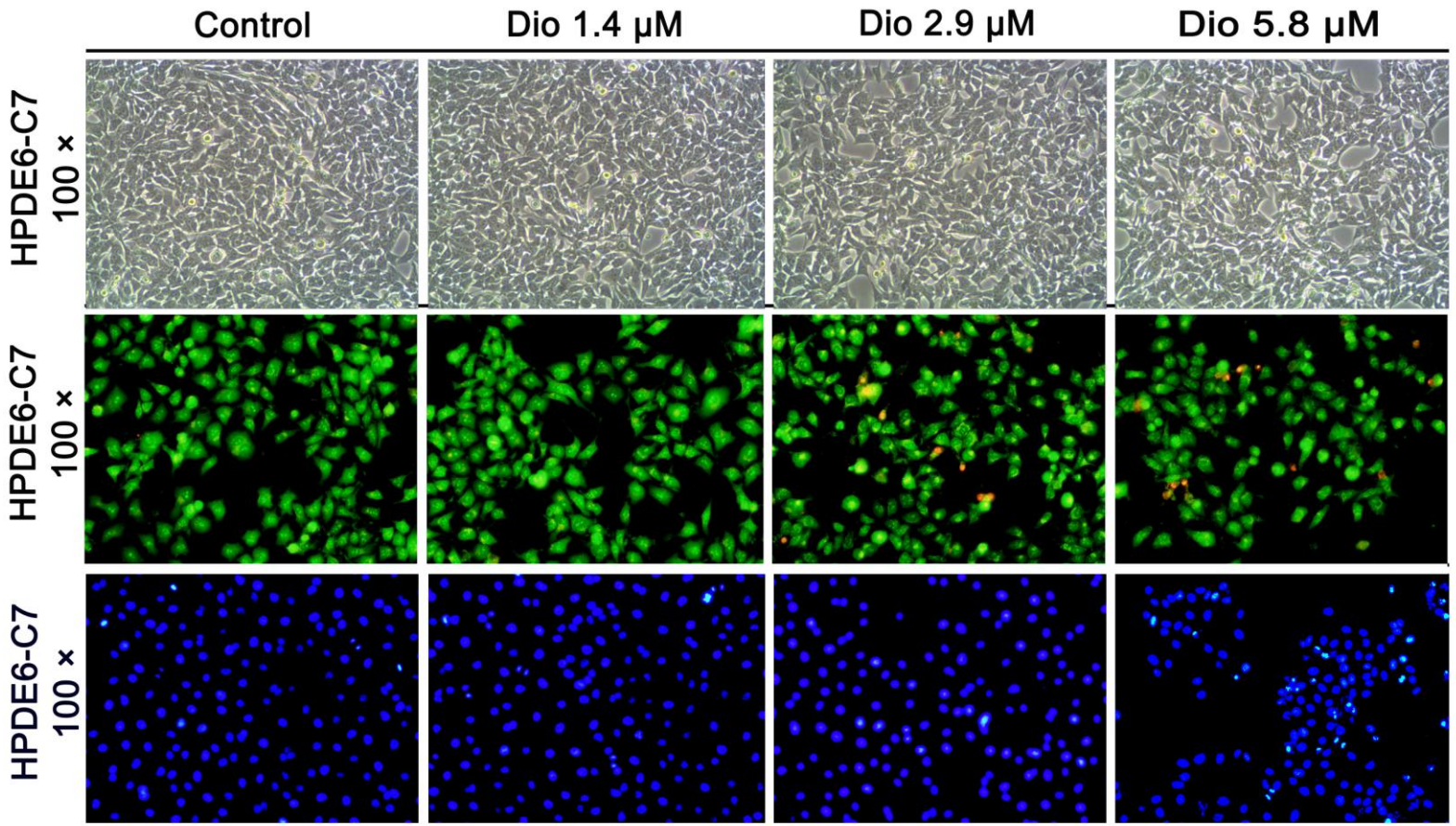
[b] T-test result between the tested samples in different groups.



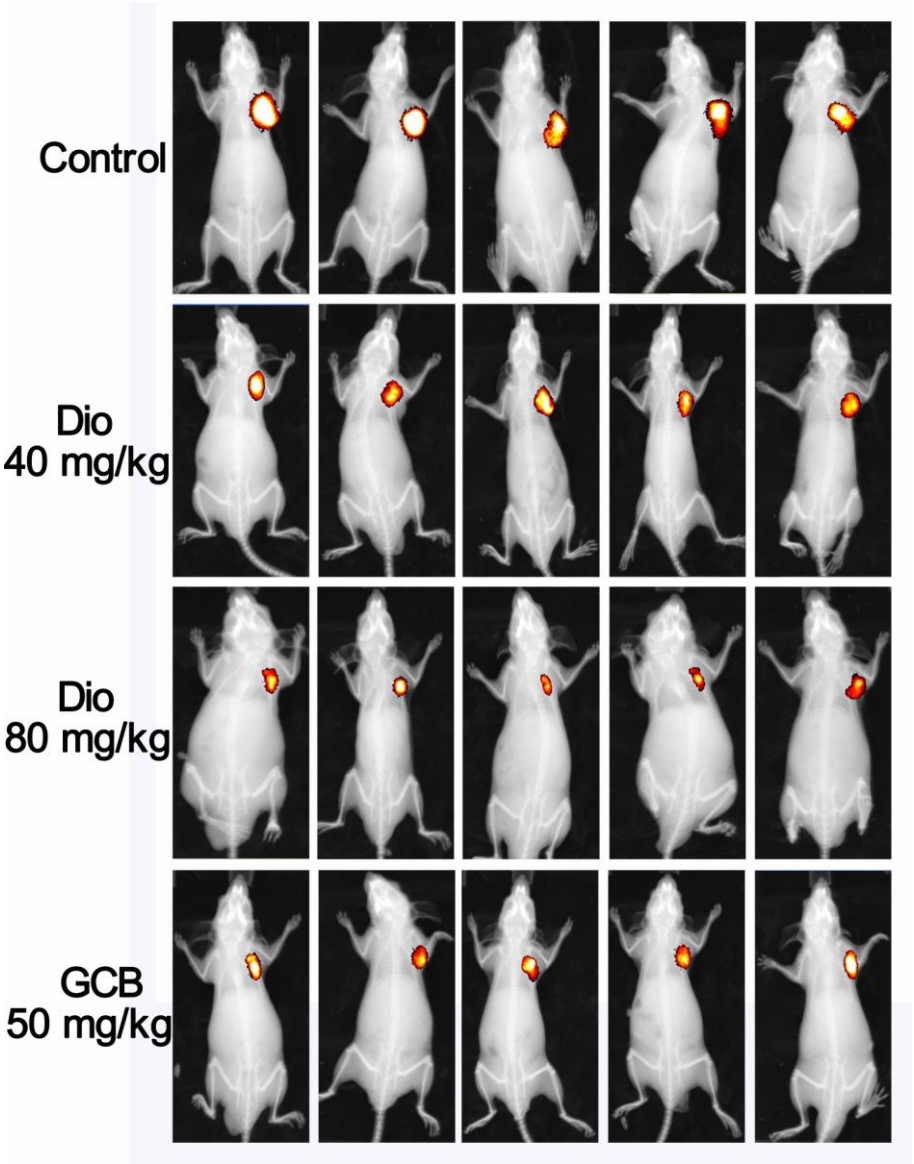
Supplemental Fig. 1. The chemical structure of dioscin.



Supplemental Fig. 2. TEM micrographs of ASPC-1 and PANC-1 cells without or with dioscin (5.8 μM) for 24 h in different magnifications (8000 × in the left and 30000 × in the right).

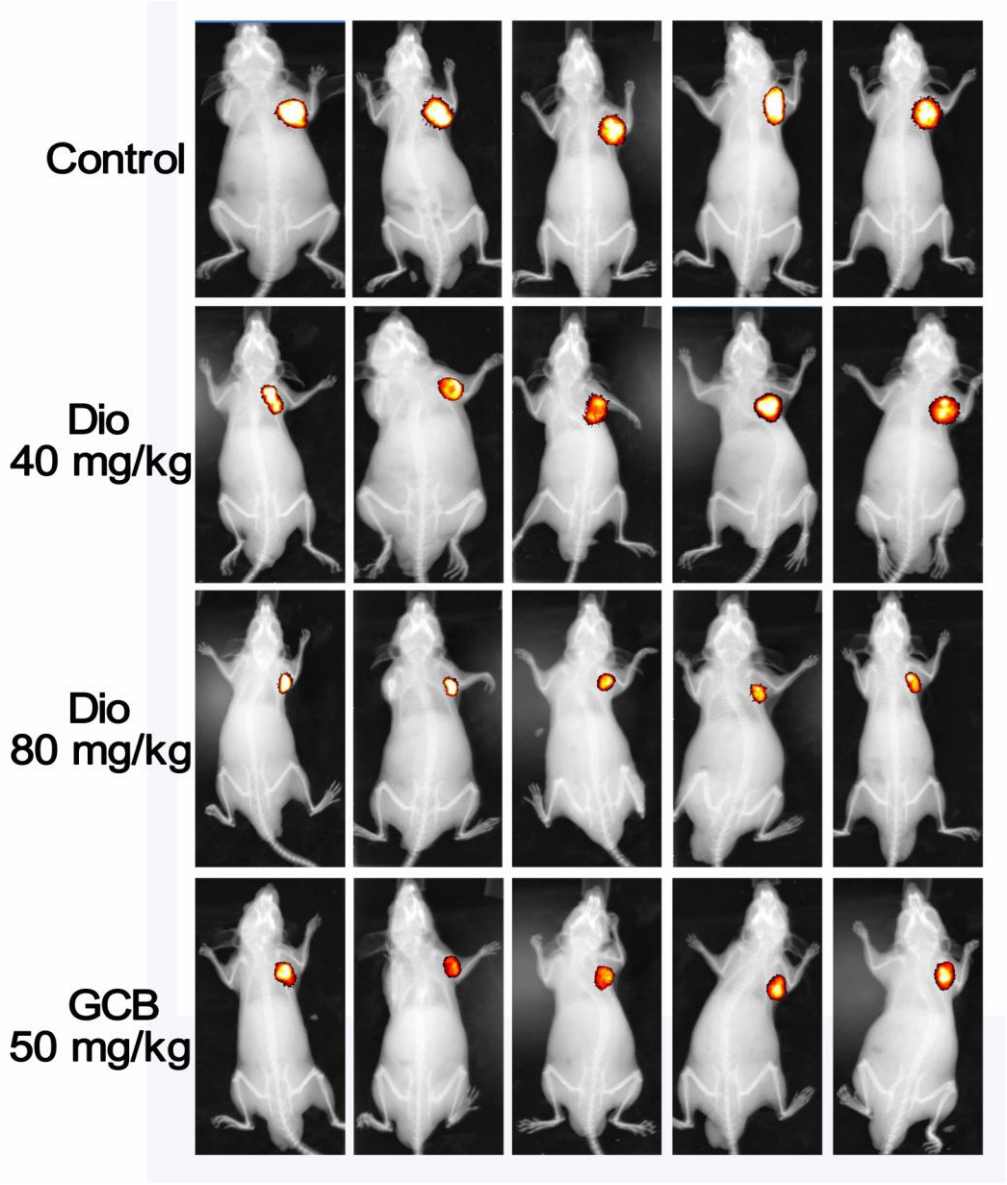


Supplemental Fig. 3. Effects of different concentrations of dioscin (1.4, 2.9 and 5.8 μM) for 24 h on HPDE6-C7 cell morphology and structure (100× magnification).

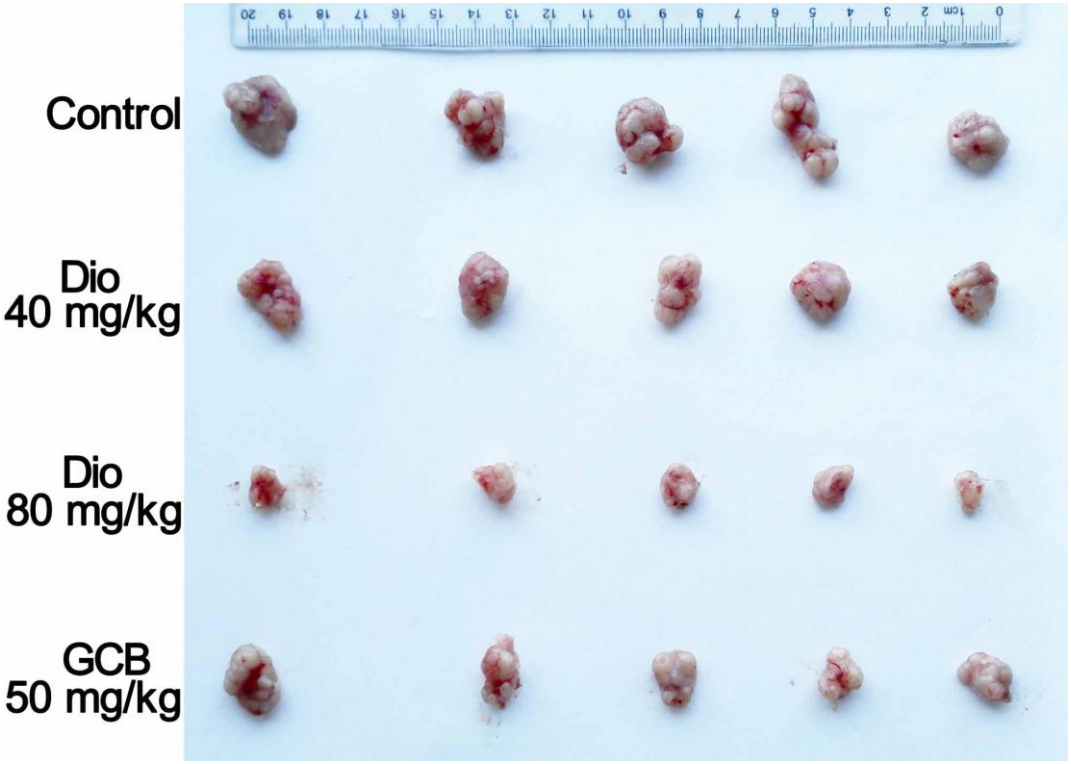


Supplemental Fig. 4. The raw bioluminescence images of the ASPC-1 cells-inoculated mice (n = 5).

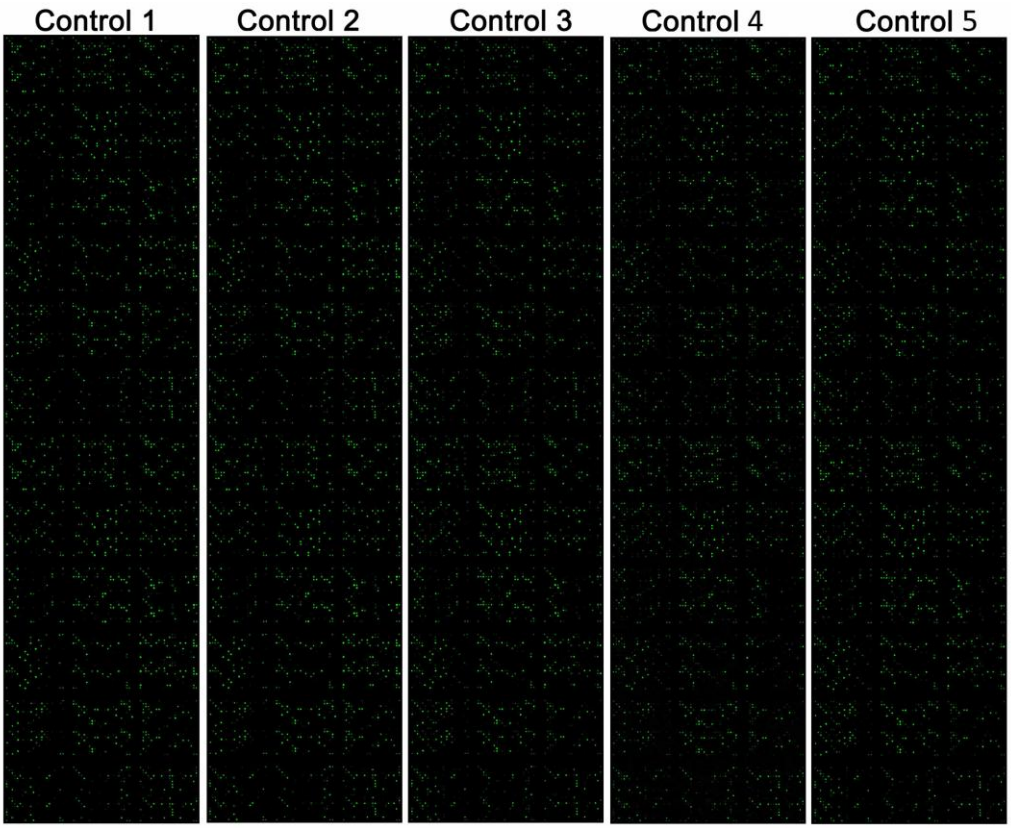
Supplemental Fig. 5. The raw tumors images of the ASPC-1 cells-inoculated mice (n = 5).



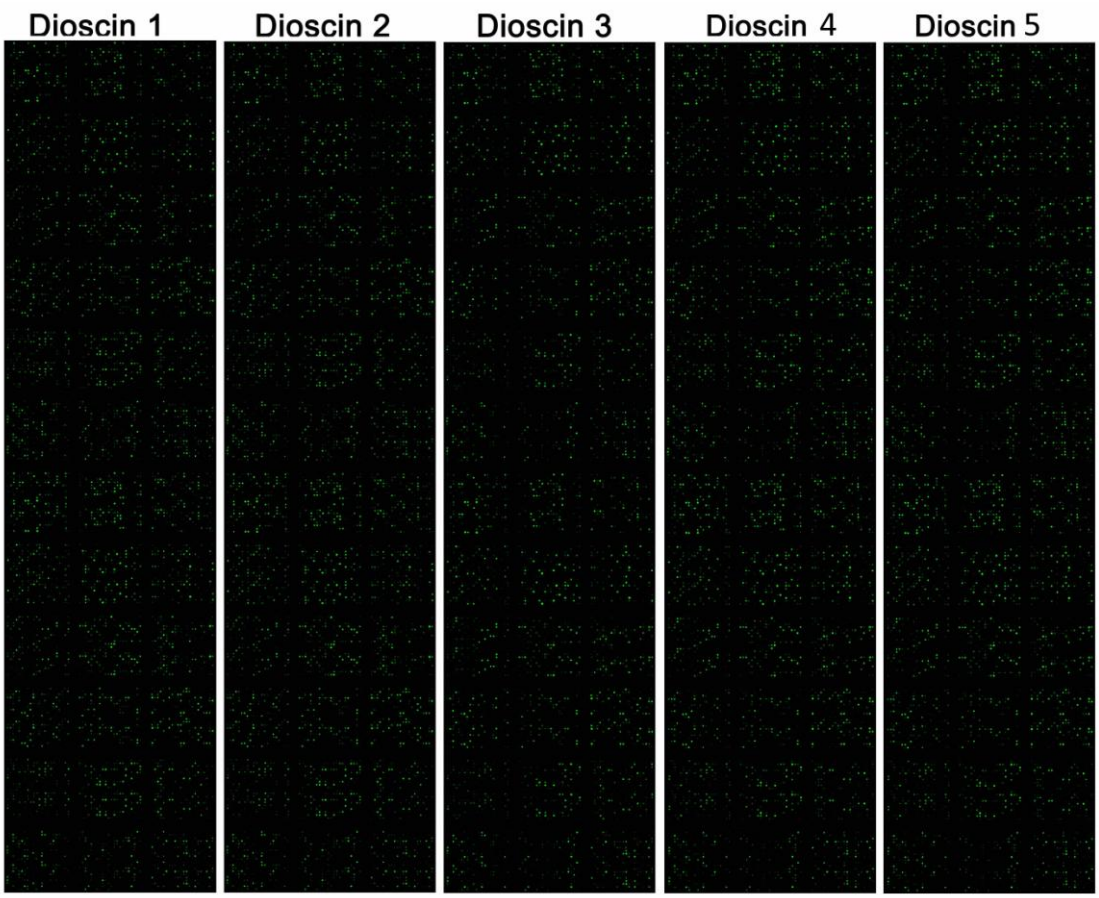
Supplemental Fig. 6. The raw bioluminescence images of the PANC-1 cells-inoculated mice (n = 5).



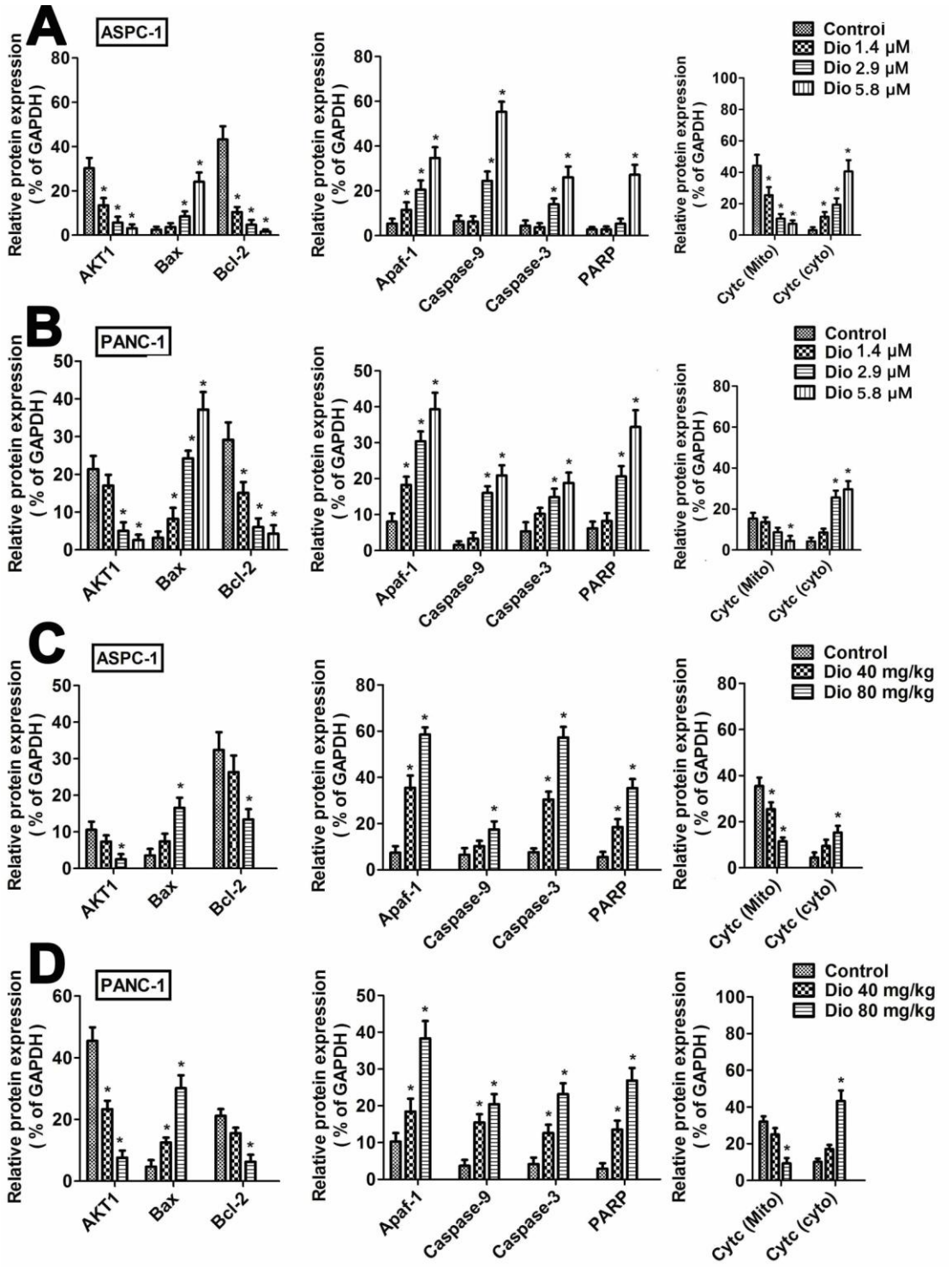
Supplemental Fig. 7. The raw tumors images of the PANC-1 cells-inoculated mice (n = 5).



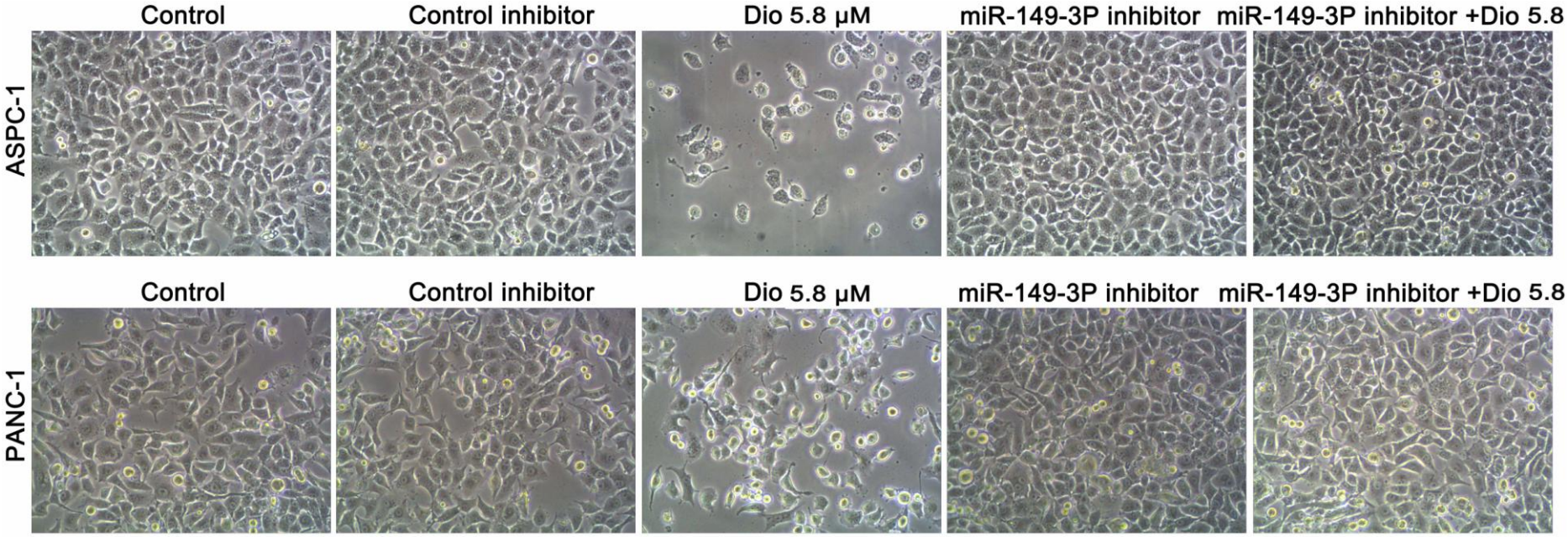
Supplemental Fig. 8. Detection of miRNAs by microarrays. Total RNAs extracted from control groups were covalently labeled with Hy3 (green channel) and hybridized to the array.



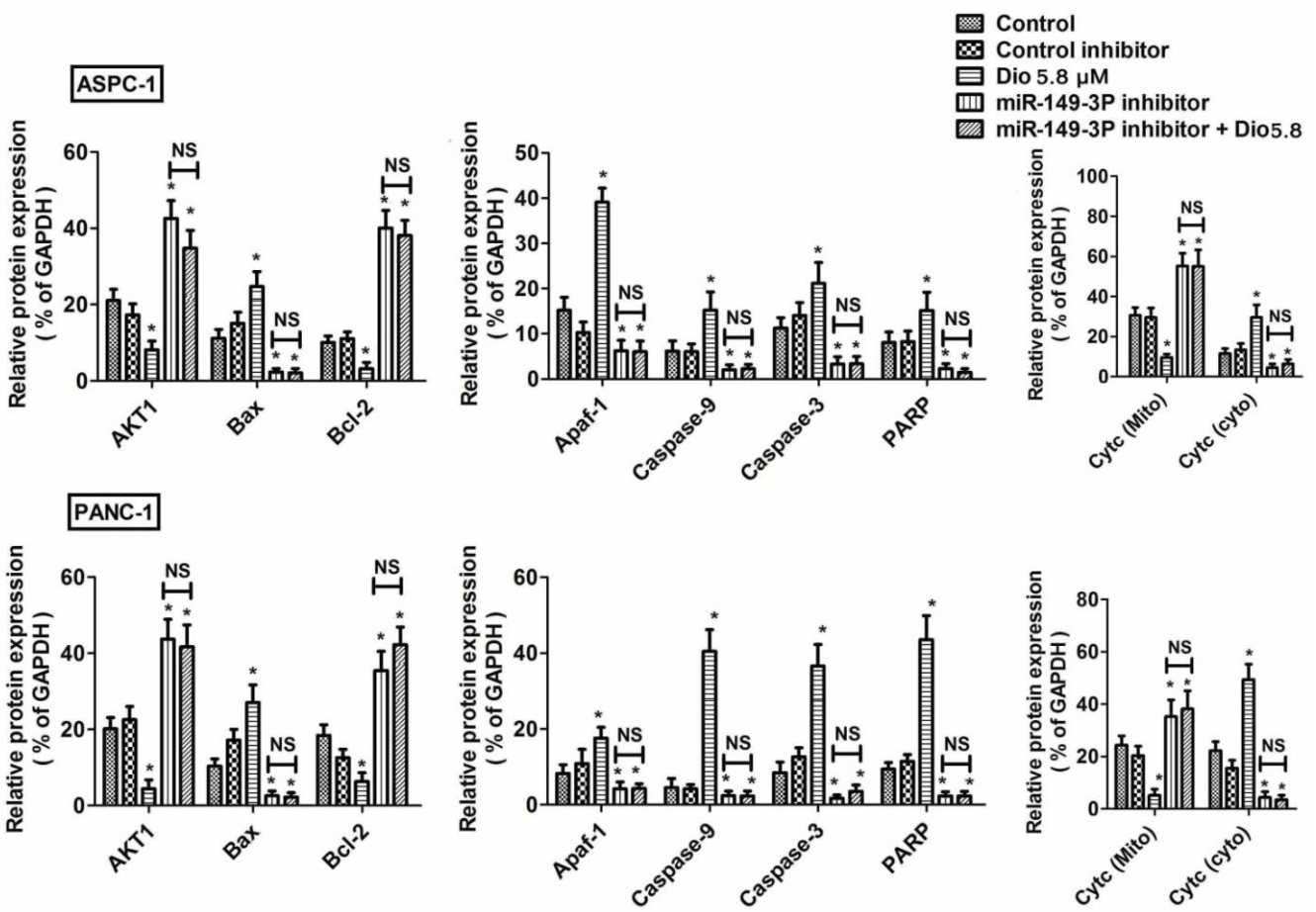
Supplemental Fig. 9. Detection of miRNAs by microarrays. Total RNAs extracted from dioscin-treated group (5.8 μM) were covalently labeled with Hy3 (green channel) and hybridized to the array.



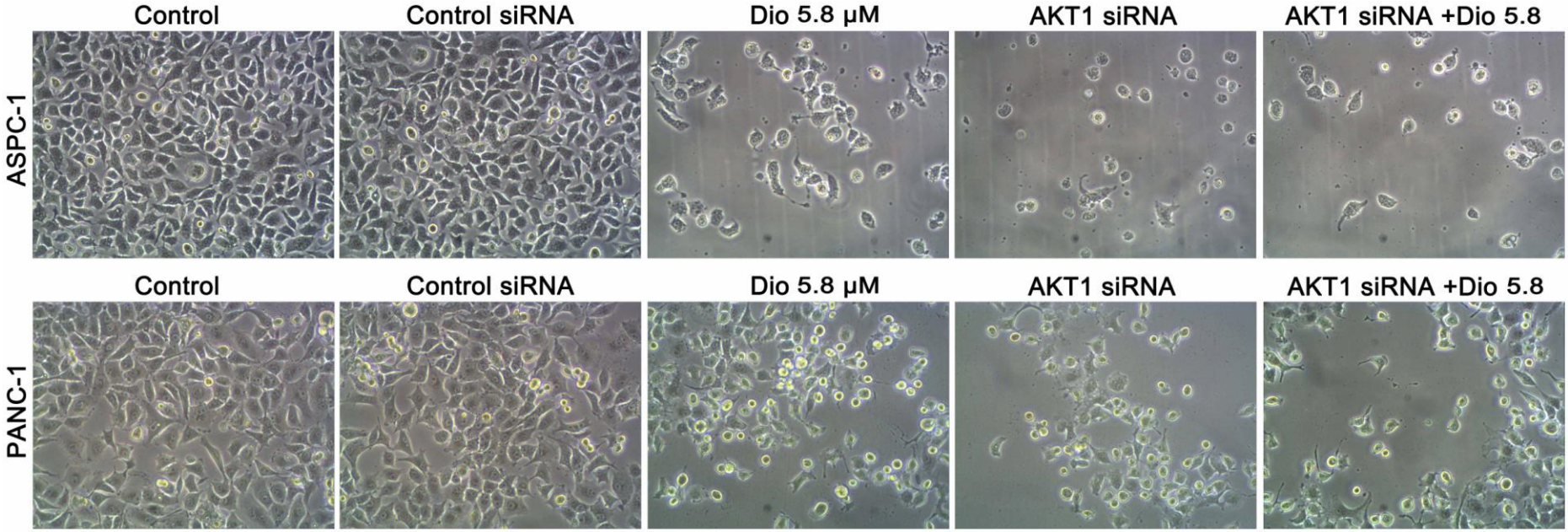
Supplemental Fig. 10. (A-D) Analytical results the protein levels of AKT1, Bax, Bcl-2, Apaf-1, Cleaved caspase-3/9, cleaved PARP and Cytochrome c treated by dioscin *in vitro* and *in vivo*. Data are presented as the mean ± S.D. (n = 5). *P < 0.05 compared with control groups.



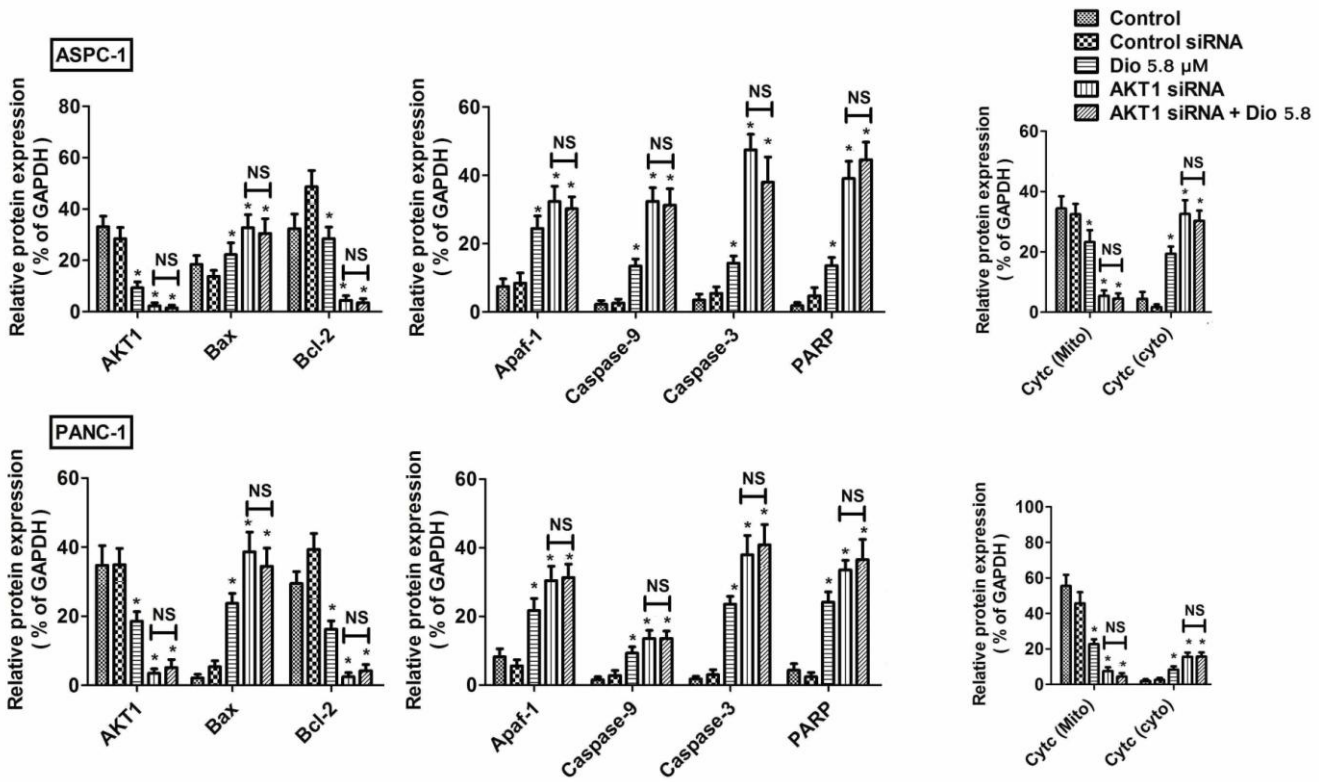
Supplemental Fig. 11. Effects of dioscin on cellular morphology and structure of ASPC-1 and PANC-1 cells by bright image (×100, magnification) investigation with or without transfecting miR-149-3P inhibitor *in vitro*.



Supplemental Fig. 12. Analytical results the protein levels of AKT1, Bax, Bcl-2, Apaf-1, Cleaved caspase-3⁄9, cleaved PARP and Cytochrome c after treated with miR-149-3P inhibitor in ASPC-1 and PANC-1 cells. Data are presented as the mean ± S.D. (n = 5). *P < 0.05 compared with control inhibitor group; NS, not significant.



Supplemental Fig. 13. Effects of dioscin on cellular morphologies and structures of ASPC-1 and PANC-1 cells by bright image (×100, magnification) investigation with or without transfecting AKT1 siRNA *in vitro*.



Supplemental Fig. 14. Analytical results the protein levels of AKT1, Bax, Bcl-2, Apaf-1, Cleaved caspase-3/9, cleaved PARP, Cytochrome c after treated with AKT1 siRNA in ASPC-1 and PANC-1 cells. Data are presented as the mean ± S.D. (n = 5). *P < 0.05 compared with control siRNA group; NS, not significant.