

Supplementary Figures

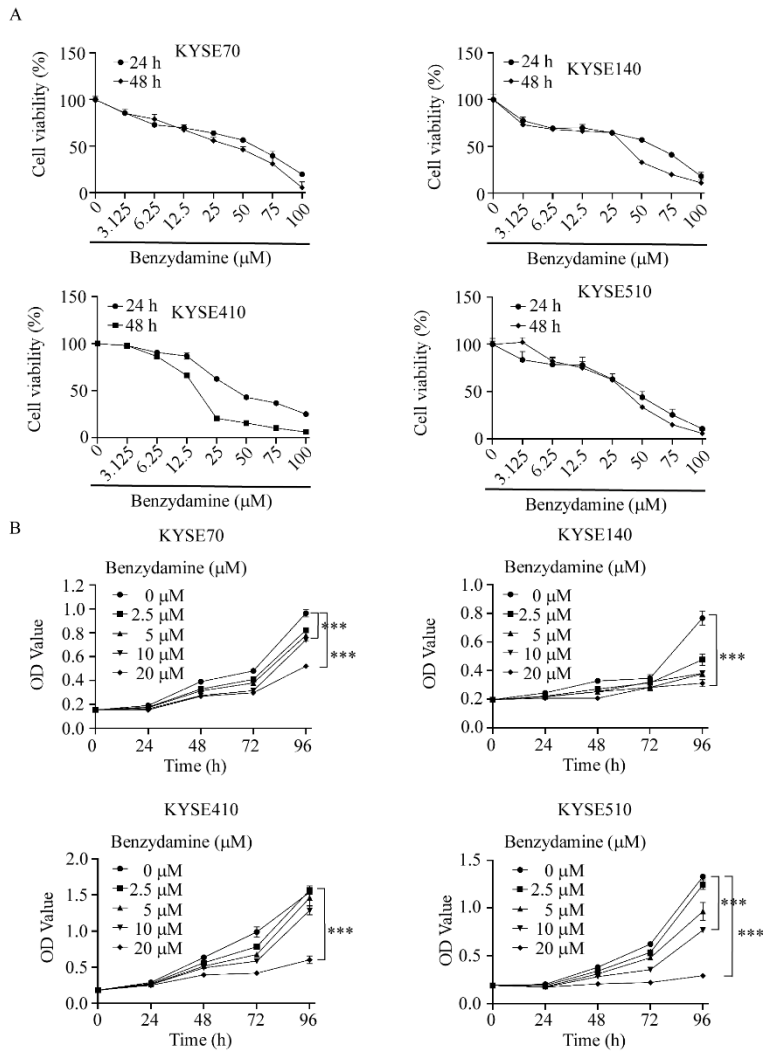


Fig.S1 Benzydamine suppressed the proliferation of ESCC cells. (a) KYSE70, KYSE140, KYSE410 and KYSE510 cells were treated with benzydamine for 24 and 48 h. Cell viability was measured by MTT assay. (b) Effects of benzydamine on the proliferation of KYSE70, KYSE140, KYSE410 and KYSE510 cells treated with various doses of benzydamine for 24, 48, 72, and 96 h.

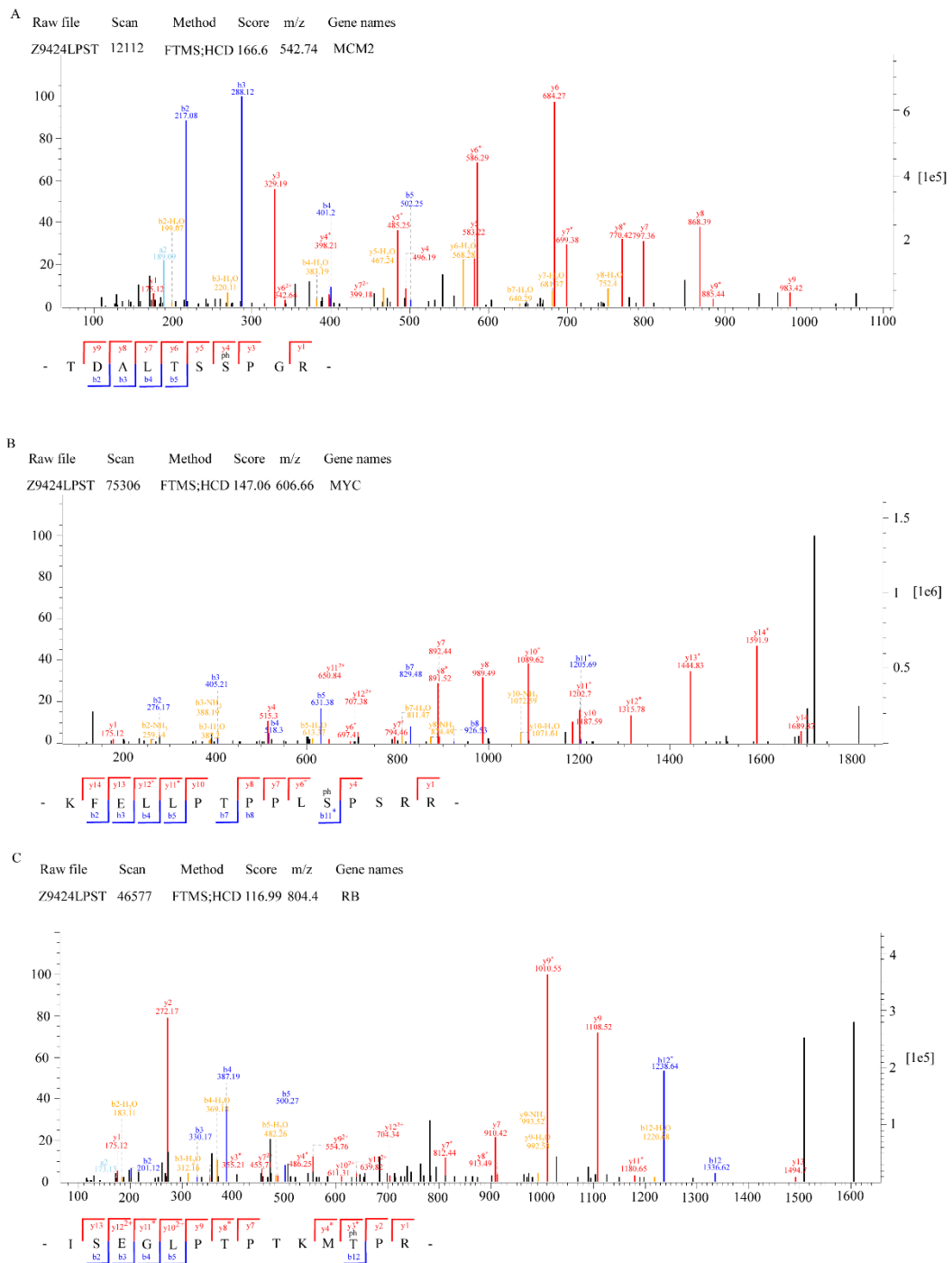


Fig. S2 Interpreted MS/MS spectra demonstrating the phosphorylation of the indicated sites in MCM2, c-Myc and Rb. The data were representative of at least three independent experiments.

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		↓			↓ ↓					
CDK2 (H.sapiens)	76	LYLV	F	EFLHQ	85	141	IKLAD	F	GLARAFGVP	155
CDK2 (B.taurus)	76	LYLV	F	EFLHQ	85	141	IKLAD	F	GLARAFGVP	155
CDK2 (M.auratus)	76	LYLV	F	ELLHQ	85	141	IKLAD	F	GLARAFGVP	155
CDK2 (D.melanogaster)	80	LYMI	F	EYLN	89	144	IKLAD	F	GLARAFNVP	158
CDK2 (X.laevis)	76	LYLV	F	EFLNQ	85	141	IKLAD	F	GLARAFGVP	155
CDK2 (M.musculus)	76	LYLV	F	EFLHQ	85	141	IKLAD	F	GLARAFGVP	155
CDK2 (C.griseus)	76	LYLV	F	EFLHQ	85	141	IKLAD	F	GLARAFGVP	155
CDK2 (R.norvegicus)	76	LYLV	F	EFLHQ	85	141	IKLAD	F	GLARAFGVP	155

Fig. S3. Sequence alignments of CDK2 phosphorylation consensus among different species.

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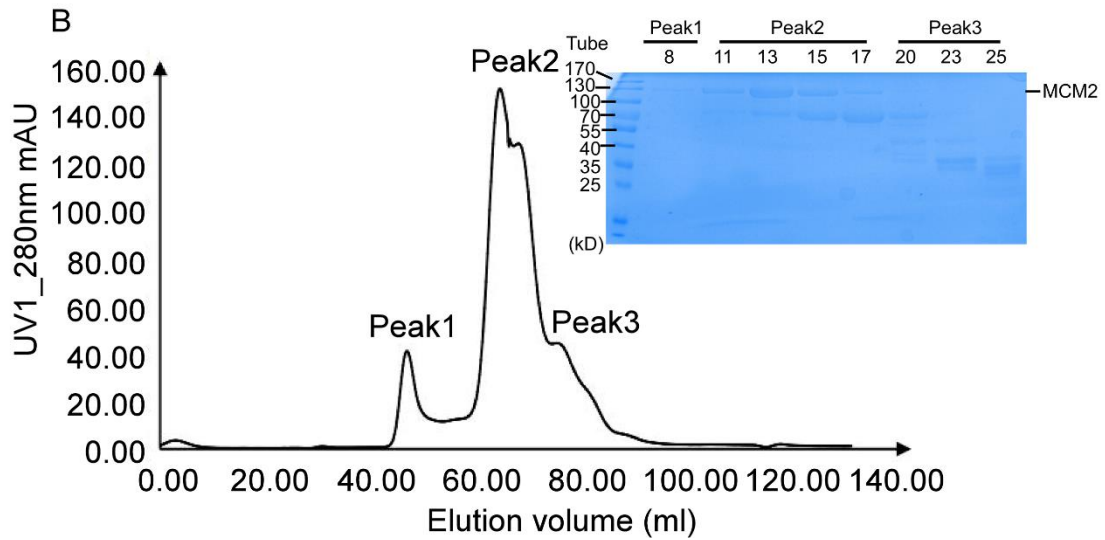
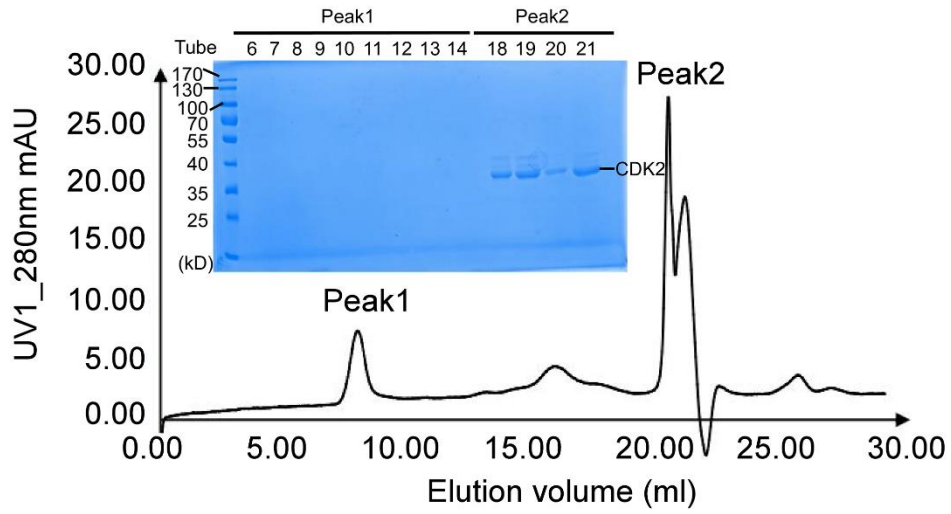


Fig. S4. Purification of the CDK2 and MCM2 proteins by size-exclusion chromatography. (a) The size-exclusion chromatography profile of CDK2 protein was presented. The purity of CDK2 protein was verified by SDS-PAGE. (b) The size-exclusion chromatography profile of the MCM2 protein. The purity of MCM2 protein was verified by SDS-PAGE.

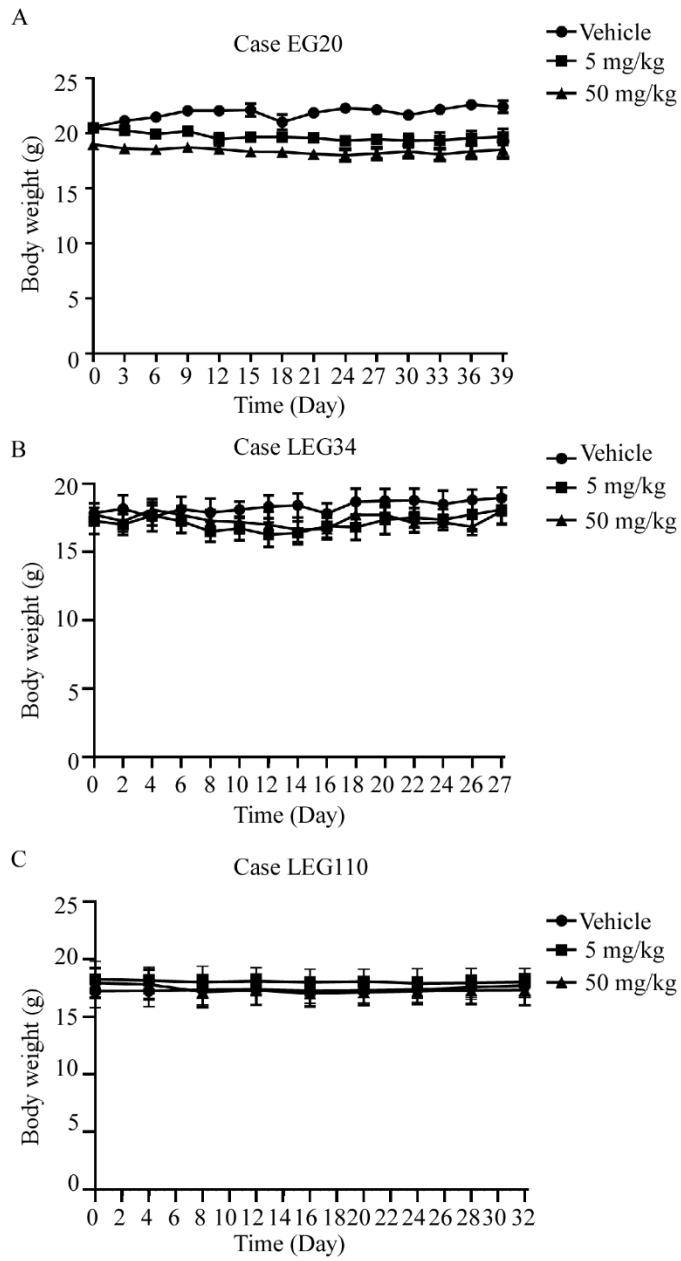


Fig. S5. The body weights of mice treated with benzydamine (5 or 50 mg/kg) were measured weekly.

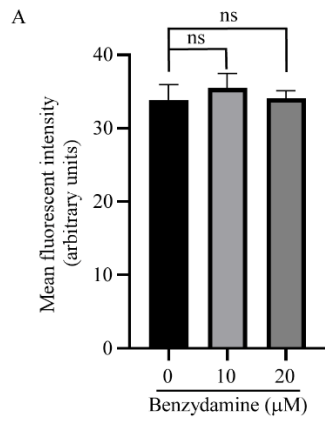


Fig. S6. Detection of COX-2 enzyme activity after benzydamine treatment by COX-2 inhibitor screening kit.

Table S1 List of downregulated kinase activity.

ID	E-ratio	X ²	P
TLK2	0.32	19.826	8.5E-06
NEK6	0.376	17.327	3.1E-05
MAPK4	0.614	14.616	0.00013
CDK3	0.686	13.582	0.00023
PLK1	0.54	13.027	0.00031
ATM	0.562	12.862	0.00034
MAPK9	0.747	11.087	0.00087
CDK1	0.755	11.06	0.00088
CSNK2A2	0.588	10.618	0.00112
UHMK1	0.733	10.083	0.0015
HIPK3	0.765	9.669	0.00187
MTOR	0.741	9.651	0.00189
MARK1	0.507	9.573	0.00197
GRK5	0.489	9.26	0.00234
MAPK14	0.764	9.184	0.00244
MAPK10	0.768	9.108	0.00254
MAPK8	0.771	8.894	0.00286
CDK9	0.779	8.836	0.00295
CDK4	0.773	8.694	0.00319
MAPK3	0.775	8.579	0.0034
LRRK2	0.403	8.498	0.00356
STK4	0.533	8.478	0.00359
CDK12	0.777	8.373	0.00381
GRK4	0.511	8.364	0.00383
CDK6	0.778	8.306	0.00395
MAPK1	0.78	8.097	0.00443
CDK2	0.781	8.07	0.0045
CDK5	0.791	7.34	0.00674
DYRK2	0.792	7.123	0.00761
NEK2	0.501	6.746	0.0094
MAPK12	0.783	6.317	0.01196
TTBK1	0.744	6.256	0.01238
NLK	0.795	5.895	0.01518
MAP2K7	0.551	5.892	0.01521
TTK	0.594	5.556	0.01842
MYLK	0.496	5.516	0.01884
PAK3	0.655	5.219	0.02234
IKBKB	0.71	5.152	0.02322
MAPK11	0.807	4.845	0.02773
PLK2	0.735	4.401	0.03592
RIPK1	0.597	4.339	0.03725
HIPK1	0.822	4.284	0.03847

FAM20C	0.502	4.003	0.04542
PLK3	0.695	3.922	0.04766

Table S2 List of molecular docking.

Target	Common name	Target class	Estimated (kcal/mol)	ΔG
Cyclin-dependent kinase 2	CDK2	Kinase	-8.04	
Protein kinase C theta	PRKCQ	Kinase	-7.99	
TGF-beta receptor type I	TGFB1	Kinase	-7.98	
Protein kinase C beta	PRKCB	Kinase	-7.97	
Tyrosine-protein kinase receptor FLT3	FLT3	Kinase	-7.97	
Serine/threonine-protein kinase PIM1	PIM1	Kinase	-7.96	
Protein kinase C (PKC)	PRKCZ	Kinase	-7.94	
Tyrosine-protein kinase JAK2	JAK2	Kinase	-7.92	
Serine/threonine-protein kinase PIM3	PIM3	Kinase	-7.85	
Serine/threonine-protein kinase Aurora-A	AURKA	Kinase	-7.77	
Protein kinase C eta	PRKCH	Kinase	-7.76	
Epidermal growth factor receptor erbB1	EGFR	Kinase	-7.67	
Protein kinase C alpha	PRKCA	Kinase	-7.65	
PDZ-binding kinase	PBK	Kinase	-7.63	
ALK tyrosine kinase receptor	ALK	Kinase	-7.5	
Protein kinase C epsilon	PRKCE	Kinase	-7.29	
Insulin receptor	INSR	Kinase	-7.12	
Protein kinase C delta	PRKCD	Kinase	-6.97	
Protein kinase C gamma	PRKCG	Kinase	-6.97	