

Cell cycle arrest, an important mechanism of action of Compound Kushen injection, prevents colorectal cancer: network pharmacology analysis and experimental validation

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Supplementary Table S1 A list of target genes and corresponding compounds in the treatment of colorectal cancer with CKI.

PubChem CID	Compound	Herb	Target	Uniprot ID
12442899	Sophoranol	Kushen	IRF1	P10914
509245	sophoraflavanone B	Kushen	IL10	P22301
259846	lupeol	Kushen	MDM2	Q00987
			MTOR	P42345
			MDM2	Q00987
115012	soyasapogenol B	Kushen	MTOR	P42345
			ANP32A	P39687
			MDM2	Q00987
3082765	dehydromiltirone	Kushen	ANP32A	P39687
			EGF	P01133
			MTOR	P42345
			MDM2	Q00987
102073915	kusulactone	Kushen	MTOR	P42345
			ANP32A	P39687
			MDM2	Q00987
91572	Phaseolin	Kushen	MTOR	P42345
			EGF	P01133
			ANP32A	P39687
91510	maackiain	Kushen	ERBB2	P04626
10854625	kushenol S	Kushen	ERBB2	P04626
5319307	matsukaze lactone	Kushen	ERBB2	P04626
5281611	Resokaempferol	Kushen	HOXB13	Q92826
			MAP2K2	P36507
42607818	sophoranochromene	Kushen	HOXB13	Q92826
91466	matrine	Kushen	UNG	P13051
			PTPRJ	Q12913
10659287	(-)-14 β -hydroxymatrine	Kushen	UNG	P13051
			PTPRJ	Q12913
114850	Oxymatrine	Kushen	UNG	P13051
			PTPRJ	Q12913
165549	sophoridine	Kushen	UNG	P13051
			PTPRJ	Q12913
5271984	isomatrine	Kushen	UNG	P13051
			PTPRJ	Q12913
115269	sophocarpine	Kushen	UNG	P13051
			PTPRJ	Q12913
24721085	Oxysophocarpine	Kushen	UNG	P13051
			PTPRJ	Q12913
126455735	(-)-leontalbinine N-oxide	Kushen	UNG	P13051
			PTPRJ	Q12913

169014	sophoramine	Kushen	UNG PTPRJ	P13051 Q12913
3041752	lehmannine	Kushen	UNG PTPRJ	P13051 Q12913
10742453	kushenol P	Kushen	UNG MPO	P13051 P05164
10835998	kushenol R	Kushen	UNG	P13051
10598514	kushenol T	Kushen	UNG	P13051
44563198	kurarinol	Kushen	UNG	P13051
72936	(-)-sophoraflavanone G	Kushen	UNG	P13051
10599228	kushenol X	Kushen	UNG	P13051
381851	kushenol N	Kushen	UNG	P13051
133561937	2'-methoxykurarinone	Kushen	UNG	P13051
5275227	leachianone G	Kushen	UNG	P13051
20056309	sophoraflavoside I	Kushen	UNG	P13051
68406	Octacosanol	Kushen	UNG	P13051
14104288	3,7,4'-Trihydroxy-5-methoxy- 8-prenylflavanone	Kushen	UNG	P13051
15953774	2-(2,4-Dihydroxy-5- prenylphenyl)-5,6- methylenedioxybenzofuran	Kushen	UNG	P13051
129716399	8-Isopentenyl-kaempferol	Kushen	UNG	P13051
44584550	koumidine	Kushen	UNG	P13051
101297615	normacusine b	Kushen	UNG	P13051
442822	sophoraisoflavanone a	Kushen	UNG	P13051
15385683	(+)-14 α -hydroxymatrine	Kushen Kushen	UNG PTPRJ	P13051 Q12913
14379237	5,6-dehydrolupanine	Kushen	MTOR EGF ANP32A	P42345 P01133 P39687
6326060	sophojaponicin	Kushen	MTOR DAB2	P42345 P98082
44257277	genistein-7-O-rutinoside	Kushen	TP53	P04637
5492234	lanceolarin	Kushen	TP53 EGF	P04637 P01133
623329	(-)-12-ethylsophoramine	Kushen	ANP32A PTPRJ	P39687 Q12913
3085182	Manmanine	Kushen	EGF ANP32A	P01133 P39687
14353465	4'-hydroxyisolonchocarpin	Kushen	EGF	P01133
162147	Aloperine	Kushen	EGF ANP32A	P01133 P39687
7014	sparteine	Kushen	EGF ANP32A	P01133 P39687

71317062	Genistein-7-O-glucoside	Kushen	TYMS	P04818
			MAP2K2	P36507
10496772	kushecarpin C	Kushen	PTPRJ	Q12913
73145	β -Amyrin	Kushen	ANP32A	P39687
			AURKA	O14965
442827	trifolirhizin	Kushen	DHFR	P00374
			CHEK1	O14757
			IGF1	P05019
44257441	(-)-Maackiain-3-O-glucoside	Kushen	DAB2	P98082
14841223	Trifolirhizin 6'-O-malonate	Kushen	DAB2	P98082
10982109	Specionin	Kushen	BIRC5	O15392
			PLA2G2A	P14555
21721878	kushenol L	Kushen	TP73	O15350
			AXIN1	O15169
6440079	Hexadecyl-ferulate	Kushen	PLA2G2A	P14555
15385684	(+)-9 α -hydroxymatrine	Kushen	ALK	Q9UM73
			CCND1	P24385
10495761	kushecarpin A	Kushen	TP73	O15350
14274649	5,9-dihydroxymatrine	Kushen	CTNNB1	P35222
10382239	isostrychnine	Kushen	AR	P10275
5319322	medicagol	Kushen	TOP1	P11387
101667015	epilamprolobine N-oxide	Kushen	TOP1	P11387
9576780	macrozamin	Baituling	ERBB4	Q15303
3220	Emodin	Baituling	ERBB4	Q15303
119422	Hesperetin 7-O-rutinoside	Baituling	DICER1	Q9UPY3
10168	1,8-Dihydroxy-3-carboxyanthraquinone	Baituling	DLC1	Q96QB1
346868	anthesisol	Baituling	CDKN2A	Q8N726
24836924	amylum	Baituling	MMP1	P03956
316844	isoastilbin	Baituling	DNMT1	P26358
101937309	isoengelitin	Baituling	DNMT1	P26358
			UNG	P13051
73642	Resveratrol-3-O- β -D-glucopyranoside	Baituling	DNMT1	P26358
12408	Octacosane	Baituling	MDM2	Q00987
69244670	3,3',5,5'-tetrahydroxy-4-methoxystilbene	Baituling	ERBB2	P04626
128735	Isobaimuxinol	Baituling	ERBB2	P04626
			PTPRJ	Q12913
114829	Liquiritigenin	Baituling	HOXB13	Q92826
119258	astilbin	Baituling	HOXB13	Q92826
119422	Hesperetin 7-O-rutinoside	Baituling	TP53	P04637
91439	smilagenin	Baituling	PTPRJ	Q12913
99474	diosgenin	Baituling	PTPRJ	Q12913

Supplementary Table S2 Key target protein and degree value of CKI for colorectal cancer

Target name	Degree	Target name	Degree
TP53	27	MTOR	15
CCND1	24	IGF1	14
CDKN2A	20	CHEK1	13
CTNNB1	19	AR	13
ERBB2	19	TYMS	12
MDM2	18	AURKA	12
EGF	18	DNMT1	12

Supplementary Table S3 CKI herb-compounds-candidate targets network parameters

Network parameters	Values
Numbers of nodes	118
Network density	0.030
Network diameter	8
Network heterogeneity	1.880
Average number of neighbors	3.458
Characteristic path length	3.259
Shortest paths	13806(100%)
Network centralization	0.526

Supplementary Table S4 The KEGG pathway predicted by RNA-seq validation and network pharmacology

No.	CKI Target pathways	Prediction	RNA-Seq validation	
		CRC (33)	SW620(32)	SW480(23)
1	cell cycle	√ (19)	√ (1)	√ (2)
2	Acute myeloid leukemia	√ (30)	√ (16)	
3	*Chronic myeloid leukemia	√ (15)	√ (3)	
4	*Colorectal cancer	√ (12)	√ (4)	
5	*Endometrial cancer	√ (5)	√ (20)	
6	*ErbB signaling pathway	√ (17))	√ (29)	
7	*FoxO signaling pathway	√ (14)	√ (7)	
8	*Glioma	√ (4)	√ (17)	
9	HTLV-1 infection	√ (27)	√ (8)	
10	*Melanoma	√ (8)	√ (15)	
11	*Non-small cell lung cancer	√ (6)	√ (28)	
12	*p53 signaling pathway	√ (7)	√ (6)	
13	*Pancreatic cancer	√ (13)	√ (18)	
14	*pathway in cancer	√ (3)	√ (22)	
15	*Proteoglycans in cancer	√ (9)	√ (2)	
16	*Prostate cancer	√ (1)	√ (23)	
17	*Viral carcinogenesis	√ (23)	√ (9)	
18	Basal transcription factors		√ (32)	√ (7)
19	Homologous recombination		√ (12)	√ (10)
20	mRNA surveillance pathway		√ (25)	√ (5)
21	Nucleotide excision repair		√ (26)	√ (8)

22	splicesome		√ (14)	√ (1)
23	Adherens junction	√ (33)		
24	Basal cell carcinoma	√ (28)		
25	Bladder cancer	√ (2)		
26	Central carbon metabolism in cancer	√ (20)		
27	Focal adhesion	√ (24)		
28	Hippo signaling pathways	√ (22)		
29	Hepatitis B	√ (29)		
30	HIF1 signaling pathway	√ (18)		
31	microRNAs in cancer	√ (10)		
32	PI3K-Akt signaling pathways	√ (21)		
33	Prolactin signaling pathway	√ (32)		
34	Signaling pathways regulating pluripotency of stem cells	√ (26)		
35	Thyroid cancer	√ (16)		
36	Thyroid hormone signaling pathway	√ (11)		
37	transcriptional misregulation in cancer	√ (31)		
38	Wnt signaling pathways	√ (25)		
39	Axon guidance		√ (21)	
40	Epstein-Barr virus infection		√ (30)	
41	Estrogen signaling pathway		√ (5)	
42	Insulin signaling pathway		√ (27)	
43	Lysine degradation		√ (31)	
44	Mismatch repair		√ (13)	
45	Propanoate metabolism		√ (11)	
46	Renal cell carcinoma		√ (19)	
47	small cell lung cancer		√ (10)	
48	Ubiquitin mediated proteolysis		√ (24)	
49	Amino sugar and nucleotide sugar metabolism			√ (16)
50	ABC transporters			√ (15)
51	DNA replication			√ (12)
52	Fanconi anemia pathway			√ (11)
53	Galactose metabolism			√ (22)
54	Herpes simplex infection			√ (17)
55	Influenza A			√ (20)
56	Leishmaniasis			√ (23)
57	Ovarian steroidogenesis			√ (18)
58	pertussis			√ (14)
59	Pyrimidine metabolism			√ (9)
60	Ribosome biogenesis in eukaryotes			√ (6)
61	RIG-like receptor signaling pathway			√ (13)
62	RNA transport			√ (3)
63	Toll-like receptor signaling pathway			√ (19)
64	Tuberculosis			√ (21)
65	Valine, leucine and isoleucine degradation			√ (4)

(number) represents the sequence number of signal pathways with statistical significance

Supplementary Table S5 The mainly KEGG pathway predicted of CKI anti-cancer by network pharmacology

No.	CKI targeted signal pathways	Pan-C ref. 16	CRC Our data	GC ref.20	EsC ref.17	LC ref.19
1	Bladder cancer	√ (19)	√ (2)	√ (56)	√ (28)	√ (4)
2	HIF1 signaling pathway	√ (17)	√ (18)	√ (15)	√ (21)	√ (20)

3	PI3K-Akt signaling pathways	√ (1)	√ (21)	√ (6)	√ (1)	√ (17)
4	*Prostate cancer	√ (6)	√ (1)	√ (2)	√ (5)	√ (6)
5	*Colorectal cancer	√ (15)	√ (12)	√ (17)	√ (24)	
6	*Chronic myeloid leukemia		√ (15)	√ (13)	√ (20)	√ (3)
7	*Endometrial cancer		√ (5)	√ (11)	√ (26)	√ (8)
8	*ErbB signaling pathway		√ (17)	√ (10)	√ (8)	√ (22)
9	Focal adhesion		√ (24)	√ (60)	√ (22)	√ (16)
10	*FoxO signaling pathway		√ (14)	√ (5)	√ (2)	√ (19)
11	*Glioma		√ (4)	√ (4)	√ (4)	√ (7)
12	*Melanoma		√ (8)	√ (9)	√ (18)	√ (5)
13	*Non-small cell lung cancer		√ (6)	√ (7)	√ (7)	√ (1)
14	*Pancreatic cancer		√ (13)	√ (8)	√ (19)	√ (9)
15	Hepatitis B	√ (7)	√ (29)	√ (1)		√ (13)
16	*p53 signaling pathway	√ (18)	√ (7)	√ (57)		√ (18)
17	*pathway in cancer		√ (3)	√ (3)		√ (11)
18	*Proteoglycans in cancer	√ (2)	√ (9)		√ (6)	√ (10)
19	Thyroid hormone signaling pathway	√ (9)	√ (11)	√ (50)		
20	microRNAs in cancer	√ (3)	√ (10)		√ (3)	
21	*Viral carcinogenesis		√ (23)	√ (58)	√ (23)	

Pan-C: Pan-Cancer; CRC: Colorectal Cancer; GC: Gastric Cancer; EsC: Esophageal Cancer; LC: Lung Cancer; (number) represents the sequence number of signal pathways with statistical significance

Supplementary Table S6 Sequences of primers for RT-qPCR analyses

Primer Name	Primer Sequence (5'-3')
β -actin Forward primer	5'-CATGTACGTTGCTATCCAGGC-3'
β -actin Reverse primer	5'-CTCCTTAATGTCACGCACGAT-3'
P53 Forward primer	5'-CAAGCAATGGATGATTTGATGCT-3'
P53 Reverse primer	5'-TGGGTCTTCAGTGAACCATGT-3'
P21 Forward primer	5'-TGTCCGTCAGAACCCATGC-3'
P21 Reverse primer	5'-AAAGTCGAAGTTCCATCGCTC-3'
CCND1 Forward primer	5'-GCTGCGAAGTGGAAACCATC-3'
CCND1 Reverse primer	5'-CCTCCTTCTGCACACATTTGAA-3'
CDKN2A Forward primer	5'-GATCCAGGTGGGTAGAAGGTC-3'
CDKN2A Reverse primer	5'-CCCCTGCAAACCTTCGTCCT-3'
CHEK1 Forward primer	5'-AATTGCCATGGGACCAACC-3'
CHEK1 Reverse primer	5'-CTAGAGGAGCAGAATCGATT-3'
MDM2 Forward primer	5'-GAATCATCGGACTCAGGTACATC-3'
MDM2 Reverse primer	5'-TCTGTCTACTAATTGCTCTCCT-3'

Supplementary Table S7 Antibodies or dyes used in this study

Name	Article number	Corporation name	Country	Dilution ratio
β -actin	SAB2100037	Sigma-Aldrich	USA	1:3000 (WB)
P53	21891-1-AP	Proteintech Group	USA	1:1000 (WB) 1:400 (IHC)
P21	#2947	Cell Signaling Technology	USA	1:1000 (WB)
Ki67	Ab15580	Abcam	UK	1:200 (IHC)
p-AKT	#4060	Cell Signaling Technology	USA	1:200 (IHC)
p-mTOR	#5536	Cell Signaling Technology	USA	1:200 (IHC)
p-CHEK1	#2348	Cell Signaling Technology	USA	1:100 (IHC)
HRP-labeled Rabbit Anti-Goat IgG	ZB-2306	Zhongshan Golden-bridge Biotechnology	China	1:1000 (WB)
	GK600710	Gene Tech	China	1X (IHC)

Figure S1

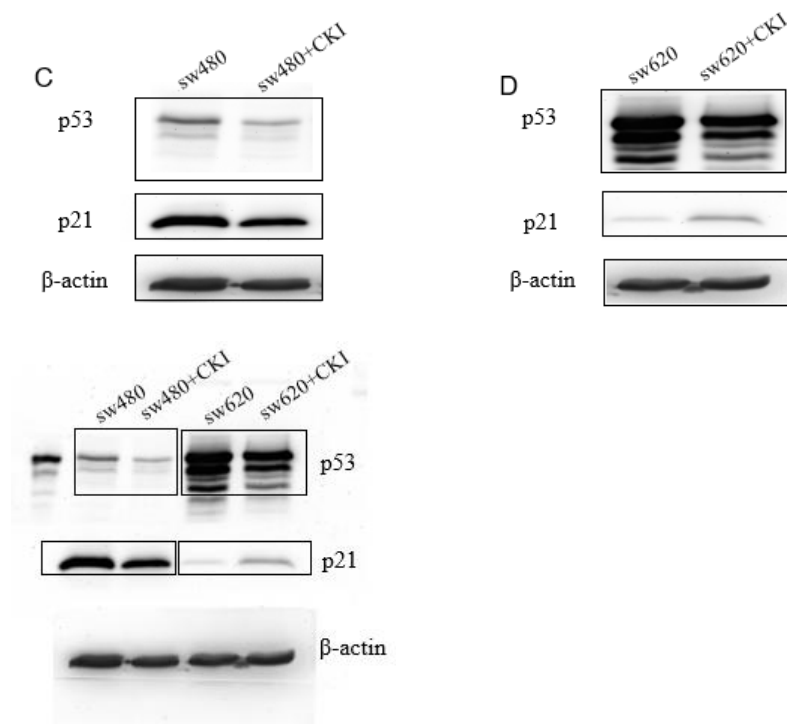


Figure S1. Full length western blot of Figure 8c, 8d