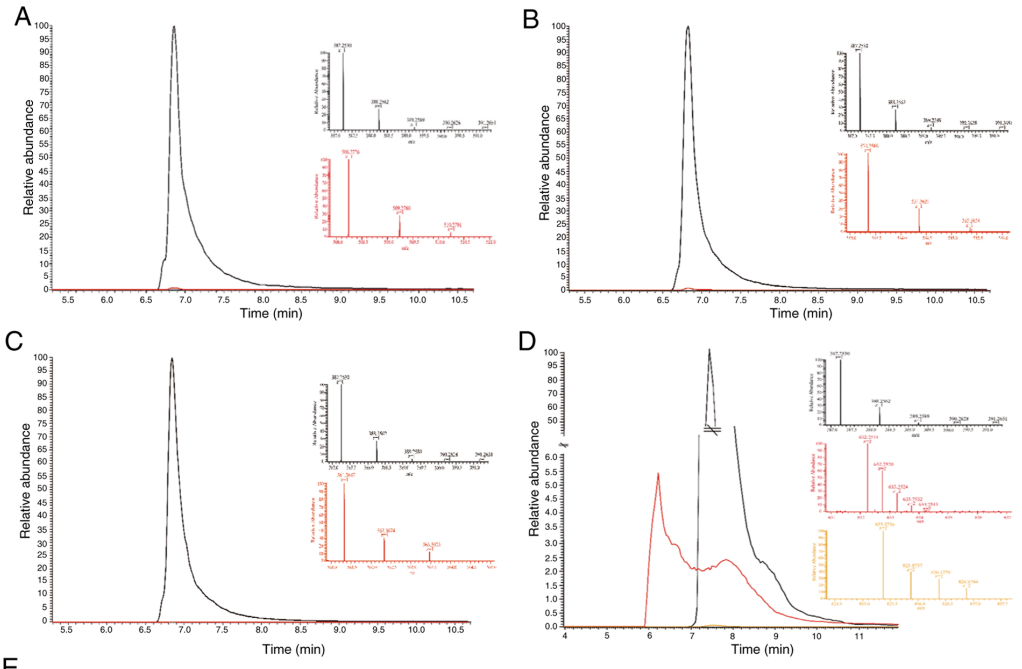
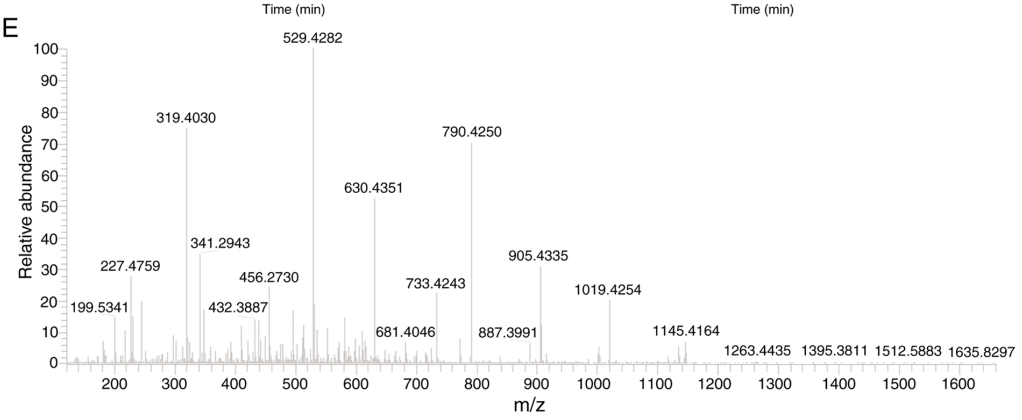
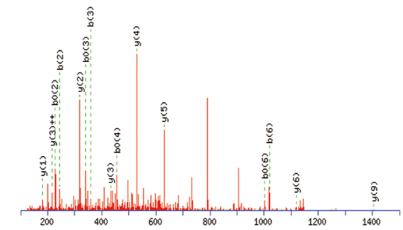
Figure S1. Bufalin reacts with peptides, and its probe CS-P1 retains antitumor activity in A549 cells. (A) XICs of bufalin (black) and cysteine-bufalin (red) determined by reversed-phase HPLC analysis. (B) XICs of bufalin black) and lysine-bufalin (red) determined by reversed-phase HPLC analysis. (C) XICs of bufalin (black) and arginine-bufalin (red) determined by reversed-phase HPLC analysis. (D) XICs of bufalin (black), peptide (red) and peptide-bufalin (orange) determined by reversed-phase HPLC analysis. (E) Secondary ion mass spectrum and mascot search results of peptide-bufalin (peptide sequence, EDNNGCTPLHY). (F) Dose-dependent inhibition of A549 cells by bufalin and CS-P1. (G) Images of intracellular localization of CS-P1 (magnification, x400). Data are presented as the mean ± SD (n=3). XIC, extracted ion chromatogram; HPLC, high-performance liquid chromatography; IC_{50}, half maximal-inhibitory concentration.

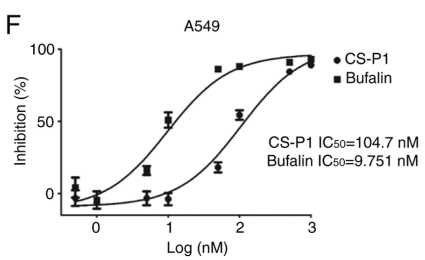






Monoisotopic mass of neutral peptide Mr(calc): 1648.7331
Variable modifications:
C6 : csp-1 (CKRSTY)
Ions score : 14 Expect: 0.039
Matches : 15/92 fragment ions using 58 most intense peaks (help)

#	b	b^{++}	b*	$b*^{++}$	b^0	b^{0++}	Seq.	y	y^{++}	y*	$y*^{++}$	y^0	y^{0++}	#
1	130.0499	65.5286			112.0393	56.5233	E							11
2	245.0768	123.0420			227.0662	114.0368	D	1520.6978	760.8525	1503.6712	752.3392	1502.6872	751.8472	10
3	359.1197	180.0635	342.0932	171.5502	341.1092	171.0582	N	1405.6708	703.3390	1388.6443	694.8258	1387.6603	694.3338	9
4	474.1467	237.5770	457.1201	229.0637	456.1361	228.5717	D	1291.6279	646.3176			1273.6173	637.3123	8
5	531.1681	266.0877	514.1416	257.5744	513.1576	257.0824	G	1176.6009	588.8041			1158.5904	579.7988	7
6	1020.4230	510.7152	1003.3965	502.2019	1002.4125	501.7099	C	1119.5795	560.2934			1101.5689	551.2881	6
7	1121.4707	561.2390	1104.4442	552.7257	1103.4602	552.2337	T	630.3246	315.6659			612.3140	306.6607	5
8	1218.5235	609.7654	1201.4969	601.2521	1200.5129	600.7601	P	529.2769	265.1421					4
9	1331.6075	666.3074	1314.5810	657.7941	1313.5970	657.3021	L	432.2241	216.6157					3
10	1468.6665	734.8369	1451.6399	726.3236	1450.6559	725.8316	H	319.1401	160.0737					2
11							Y	182.0812	91.5442					1



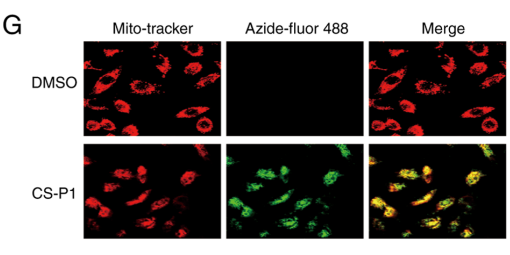


Figure S2. CS-P1 suppresses neuroblastoma cell proliferation and migration. (A) CS-P1 reduces the number and size of SK-N-BE(2) and SH-SY5Y cell colonies. (B) CS-P1 suppresses SK-N-BE(2) and SH-SY5Y cell migration (magnification, x100). Data are presented as the mean ± SD (n=3). *P<0.05, **P<0.01 vs. NC. NC, negative control.

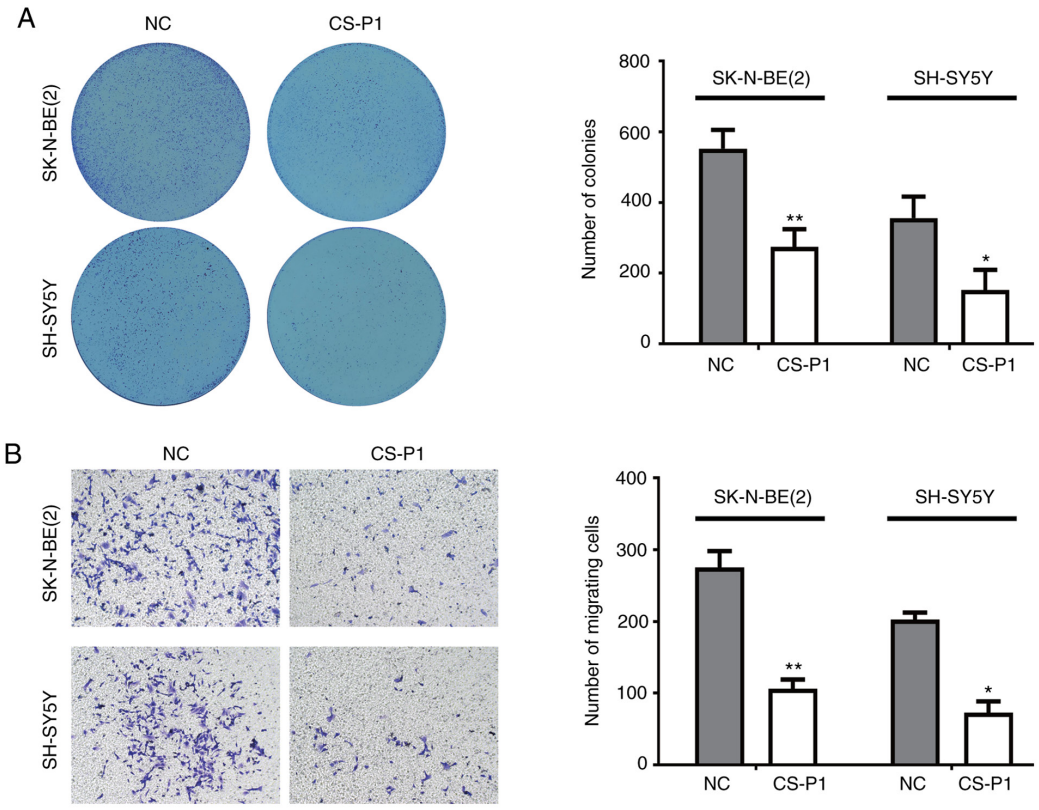


Table SI. Candidates identified by chemical proteomics.

Gene name	Protein name
CLCA1	Calcium-activated chloride channel regulator 1
DTD1	D-tyrosyl-tRNA(Tyr) deacylase 1
NBAS	Neuroblastoma-amplified sequence
NAPRT	Nicotinate phosphoribosyltransferase
PCYOX1L	Prenylcysteine oxidase-like
FAM3C	Protein FAM3C
RPIA	Ribose-5-phosphate isomerase
MRRF	Ribosome-recycling factor, mitochondrial
HSPA13	Heat shock 70 kDa protein 13
TAF6	Transcription initiation factor TFIID subunit 6
MED24	Mediator of RNA polymerase II transcription subunit 24
TFAM	Transcription factor A, mitochondrial
NOP10	H/ACA ribonucleoprotein complex subunit 3
CSPP1	Centrosome and spindle pole-associated protein 1
GINS3	DNA replication complex GINS protein PSF3
CHMP2A	Charged multivesicular body protein 2a
CERS1	Ceramide synthase 1
ARPC5L	Actin-related protein 2/3 complex subunit 5-like protein
ARHGAP17	Rho GTPase-activating protein 17
SLC31A1	High affinity copper uptake protein 1
MEMO1	Protein MEMO1
RNASEH2C	Ribonuclease H2 subunit C
GINS1	DNA replication complex GINS protein PSF1
TIGAR	Fructose-2,6-bisphosphatase TIGAR
PLD3	Phospholipase D3
EPS15	Epidermal growth factor receptor substrate 15
MYH1	Myosin-1
MVB12A	Multivesicular body subunit 12A
GTF2A2	Transcription initiation factor IIA subunit 2
PPP3R1	Calcineurin subunit B type 1
SDHD	Succinate dehydrogenase [ubiquinone] cytochrome b small subunit, mitochondrial
PON2	Serum paraoxonase/arylesterase 2
VPS11	Vacuolar protein sorting-associated protein 11 homolog
NME7	Nucleoside diphosphate kinase 7
ARPIN	Arpin
UBQLN4	Ubiquilin-4
CHTF18	Chromosome transmission fidelity protein 18 homolog
SDC1	Syndecan-1
DUS3L	tRNA-dihydrouridine(47) synthase [NAD(P)(+)]-like
SMAP	Small acidic protein
SUMF2	Sulfatase-modifying factor 2
STRIP1	Striatin-interacting protein 1
FAM98B	Protein FAM98B
HMBOX1	Homeobox-containing protein 1
MYH6	Myosin-6
CNTN1	Contactin-1
WBSCR22	Probable 18S rRNA (guanine-N(7))-methyltransferase
SGTA	Small glutamine-rich tetratricopeptide repeat-containing protein alpha
PFDN6	Prefoldin subunit 6
RAB3A	Ras-related protein Rab-3A
C4orf27	UPF0609 protein C4orf27
COX7C	Cytochrome c oxidase subunit 7C, mitochondrial
CLNS1A	Methylosome subunit pICln
RRP1B	Ribosomal RNA processing protein 1 homolog B
PRPS2	Ribose-phosphate pyrophosphokinase 2
WDR64	WD repeat-containing protein 64
PRPSAP1	Phosphoribosyl pyrophosphate synthase-associated protein 1

Table SI. Continued.

Gene name	Protein name
TMEM126A	Transmembrane protein 126A
GID8	Glucose-induced degradation protein 8 homolog
CDS2	Phosphatidate cytidylyltransferase 2
PPIL2	Peptidyl-prolyl cis-trans isomerase-like 2
TP53BP1	Tumor suppressor p53-binding protein 1
ATP5J	ATP synthase-coupling factor 6, mitochondrial
EXOC2	Exocyst complex component 2
MRPL34	39S ribosomal protein L34, mitochondrial
PI4K2A	Phosphatidylinositol 4-kinase type 2-α
RAB5B	Ras-related protein Rab-5B
PPT1	Palmitoyl-protein thioesterase 1
GOPC	Golgi-associated PDZ and coiled-coil motif-containing protein
RP2	Protein XRP2
MRPS31	28S ribosomal protein S31, mitochondrial
GABARAPL2	γ-aminobutyric acid receptor-associated protein-like 2
ADRM1	Proteasomal ubiquitin receptor ADRM1
ARID2	AT-rich interactive domain-containing protein 2
WBP2	WW domain-binding protein 2
PRRC1	Protein PRRC1
EXOSC8	Exosome complex component RRP43
MYH7	Myosin-7
TDP2	Tyrosyl-DNA phosphodiesterase 2
NOMO1	Nodal modulator 1
ATP6V1G1	V-type proton ATPase subunit G 1
M6PR	Cation-dependent mannose-6-phosphate receptor
PPIG	Peptidyl-prolyl cis-trans isomerase G
PRKAA1	5-AMP-activated protein kinase catalytic subunit α-1
COLEC11	Collectin-11
RCN2	Reticulocalbin-2
SNW1	SNW domain-containing protein 1
GOLIM4	Golgi integral membrane protein 4
DST	Dystonin
SDF2	Stromal cell-derived factor 2
XRN1	5-3 exoribonuclease 1
PAH	Phenylalanine-4-hydroxylase
ZNF22	Zinc finger protein 22
NFS1	Cysteine desulfurase, mitochondrial
SELT	Selenoprotein T
PPP1R12A	Protein phosphatase 1 regulatory subunit 12A
SRRM1	Serine/arginine repetitive matrix protein 1
PTDSS1	Phosphatidylserine synthase 1
MBLAC2	Metallo-β-lactamase domain-containing protein 2