

Figure S1. Immunoprecipitation of control IgG antibodies in A549 miCDCP1 cells expressing CDCP1 mutants, treated with rMBP-CUB proteins. (A) 6 h post-transfection with CDCP1res-F and CDCP1res-HA (A549 miCDCP1 cells) the medium was changed and 0, 5, or 10 $\mu\text{g/ml}$ rMBP-CUB2 or rMBP-CUB3 was added. Whole cell lysates were subjected to immunoblotting with anti-FLAG and anti-HA antibodies. (B) A549 miCDCP1 cell lysates treated with 10 $\mu\text{g/ml}$ rMBP-CUB2 or rMBP-CUB3 were immunoprecipitated with IgG (m) or IgG (r) antibodies. Black arrowheads indicate CDCP1, and white arrowheads indicate nonspecific bands. CDCP1, CUB domain-containing protein 1; CUB, complement 1, urchin embryonic growth factor, bone morphogenetic protein 1; rMBP, recombinant maltose binding protein; -F, FLAG-tagged; -HA, HA-tagged; IgG (m), control mouse IgG; IgG (r), control rabbit IgG.

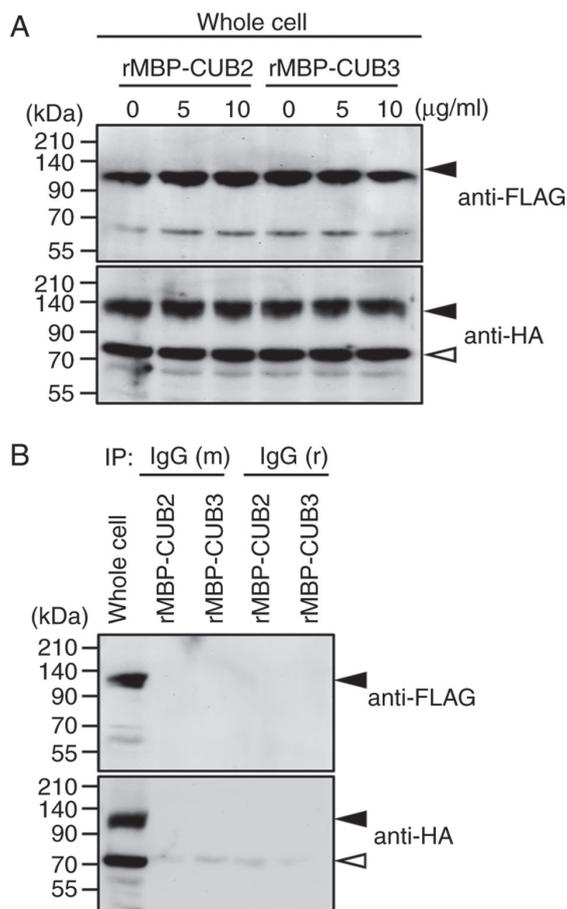


Figure S2. Cell death ratios of A549 and BxPC3 cells following addition of MBP fusion proteins. MBP fusion proteins, rMBP, rMBP-CUB2 and rMBP-CUB3, were added to the culture medium at the indicated concentrations (0, 5 or 10 $\mu\text{g/ml}$ each) and incubated with 5×10^4 cells/well in a 24-well plate. Following a 24-h incubation period, cell viability was assessed using Trypan blue staining. Assays were performed in triplicate, and the average values of three fields are shown. Mean \pm standard deviation; $n=3$ for each cell. rMBP, recombinant maltose binding protein; CUB, complement C1r/C1s, urchin embryonic growth factor, bone morphogenetic protein 1.

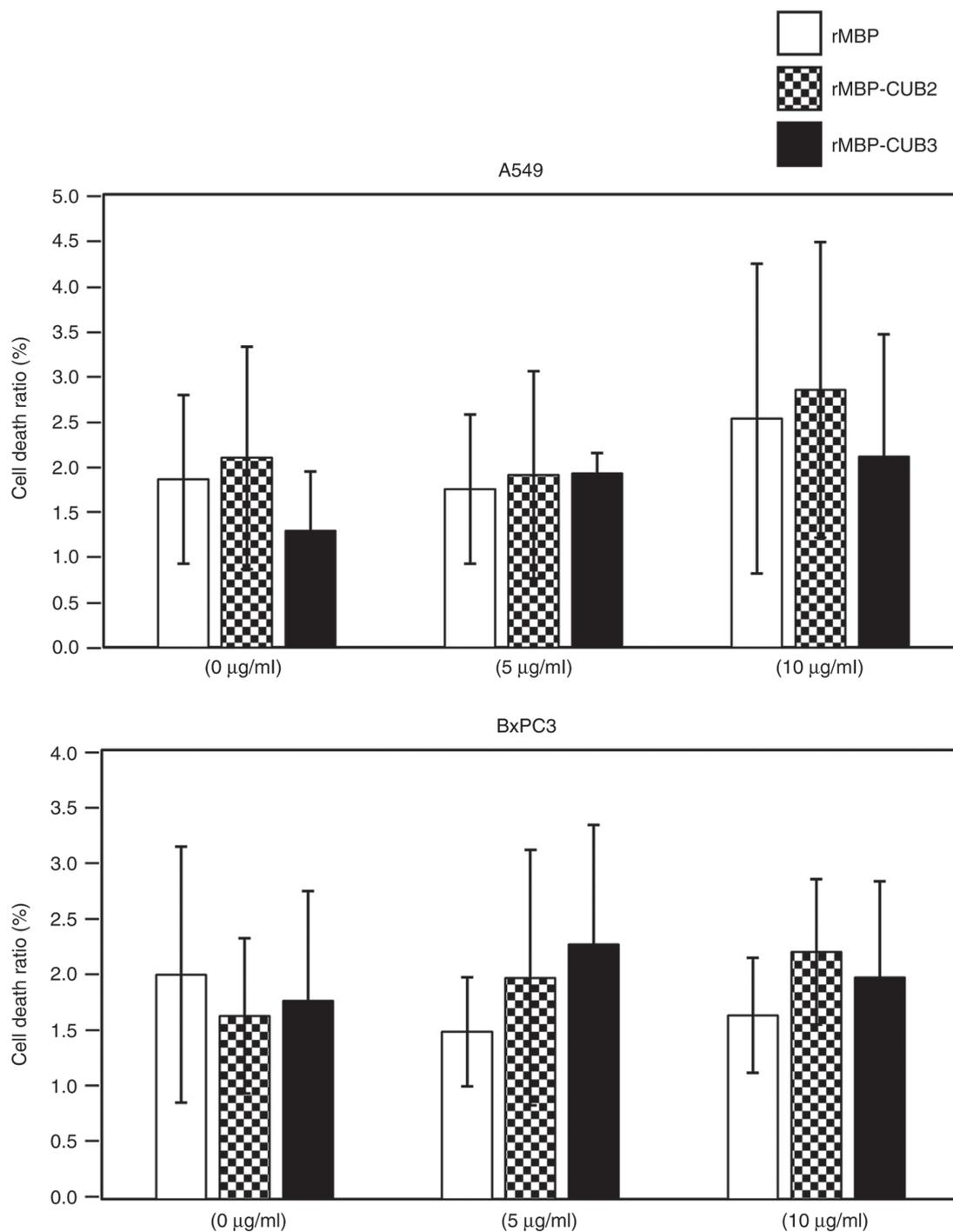


Figure S3. Effects of rMBP-CUB2 on SFK and PKC δ phosphorylation in BxPC3 cells. BxPC3 cells (1×10^5) were cultured with $10 \mu\text{g/ml}$ rMBP fusion proteins (rMBP, rMBP-CUB2, and rMBP-CUB3) for 24 h. Cell lysates were subjected to western blotting using anti-p-SFK(Y416), anti-SFK, anti-p-PKC δ (Y311) and anti-PKC δ antibodies. Proteins were quantified using ImageJ, version 1.50i. Mean \pm standard deviation; $n=3$ for each sample; * $P<0.05$, tukey's test. rMBP, recombinant maltose binding protein; CUB, complement C1r/C1s, urchin embryonic growth factor, bone morphogenetic protein 1; SFK, Src family kinase; p-, -phosphorylated; PKC δ , protein kinase C δ .

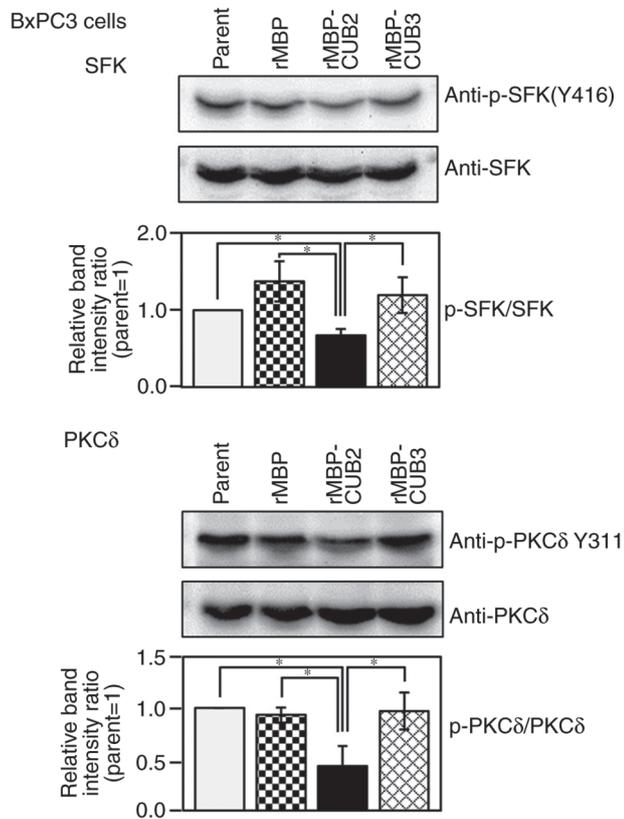


Figure S4. Scratch wound-healing assay with (A) A549 and (B) BxPC3 cells. After scratching cells and exchanging the medium, 10 $\mu\text{g/ml}$ of each MBP fusion protein (rMBP, rMBP-CUB2 and rMBP-CUB3) was added, and images were immediately captured (0 h). Cells were incubated for 12 (BxPC3 cells) or 24 h (A549 cells) in 5% CO_2 , and images were captured. Scale bar=500 μm . rMBP, recombinant maltose binding protein; CUB, complement C1r/C1s, urchin embryonic growth factor, bone morphogenetic protein 1.

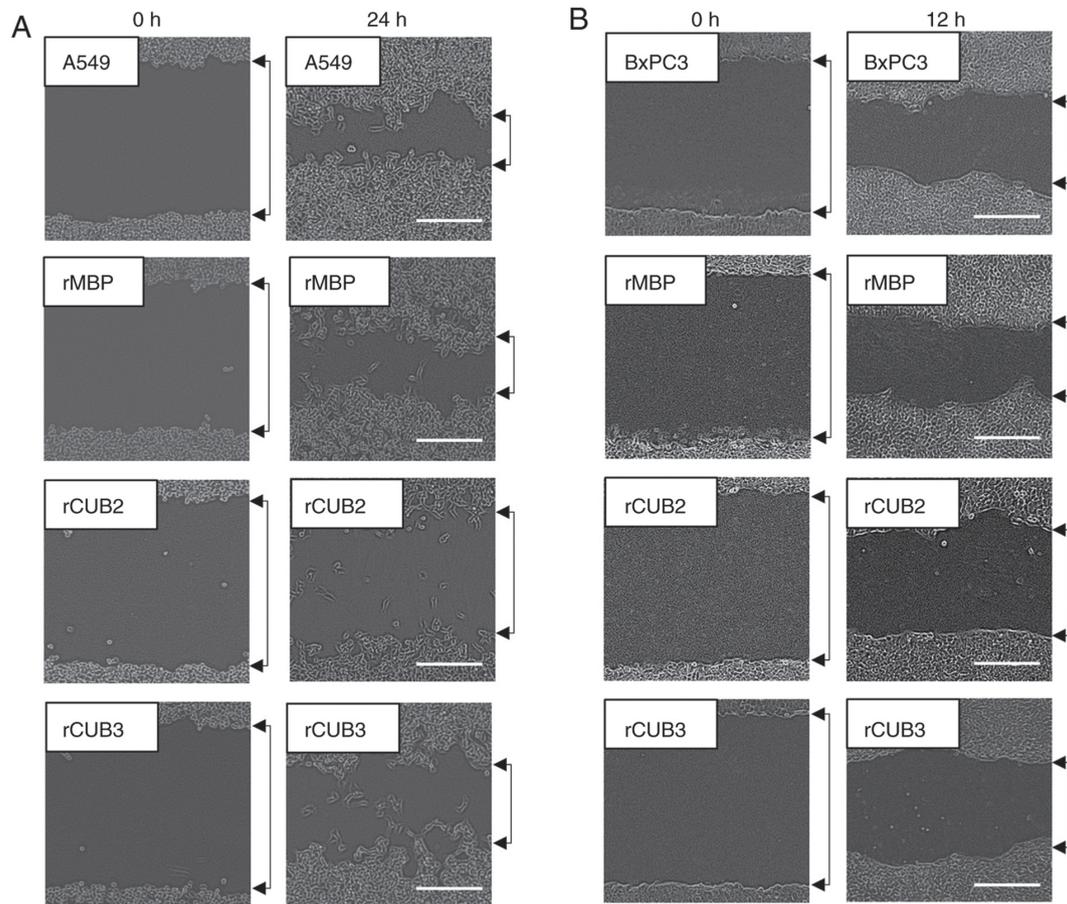


Figure S5. Structure of the CDCP1 protein (30-836 a.a. in length) with putative N-glycosylation sites (N39-N642). The 14 putative CDCP1 N-glycosylation sites are shown per a previous report (5). The CDCP1 cleavage site lies between arginine (R) at position 368 and lysine (K) at position 369, cleavage at which results in the release of a CUB1 domain-containing region. CDCP1, CUB domain-containing protein 1; CUB, complement C1r/C1s, urchin embryonic growth factor, bone morphogenetic protein 1; ECD, extracellular domain; TM, transmembrane domain; Cyto, cytoplasmic domain; a.a., amino acids.

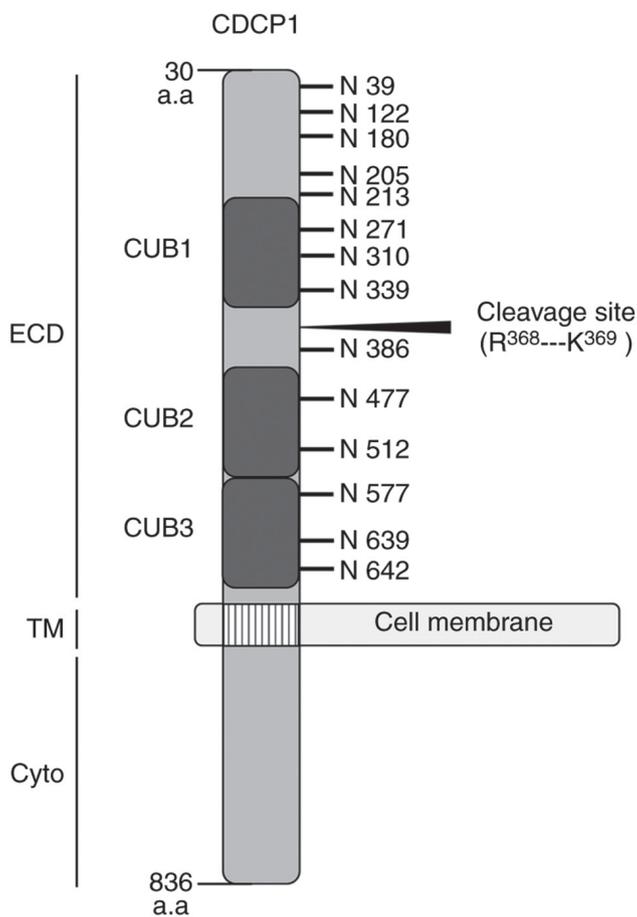


Figure S6. Amino acid sequence alignments among the human CDCP1 CUB domains. (A) Degree of homology in a.a. sequences (%) among the human CDCP1 CUB domains. (B) Black boxes indicate a.a. sequences of CUB domains aligned with CUB1 (221-348 a.a.), CUB2 (417-544 a.a.), and CUB3 (545-660 a.a.) using Clustal Omega (European Molecular Biology Laboratory-European Bioinformatics Institute). Identical amino acids are indicated with an asterisk (*); homologous sequences with high PAM 250 matrix scores (>0.5) are indicated with colons (:), and periods (.) indicate sequences with low PAM scores (≤0.5). CDCP1, CUB domain-containing protein 1; CUB, complement C1r/C1s, urchin embryonic growth factor, bone morpho-genetic protein 1; a.a., amino acids.

A

	CUB1	CUB2	CUB3
CUB1	100%	—	—
CUB2	14.84%	100%	—
CUB3	15.63%	14.06%	100%

B

CUB1	221	CIIESVFEGEGSATLMSANYPEGFP-----EDELMTWQFVVPAPHLRASVSFLNFNLS	272
CUB2	417	CTDHRYCQ-----RKSYSLQVPSDILHLPVELHDFSWKLLVPKDRLSLVLVPAQKLO	468
		* . : . * . * * . : * : * * . : * . : *	
CUB1	273	NC--ERK-EERVEYYIPGSTTNPEVFKLEDKQPGNMAG-----NFNLSLQ----GCDQD	319
CUB2	469	QHTHEKPCNTSFSYLVASAIPSQDL-YFGSFCPPGGSIKQIQVKQNISVTLRTRFAPSFQQE	527
		: * : : . * : : . : : . * * . * : : * : . : *	
CUB1	320	AQSPGILRLQFQVLVQHPQNESNKIYVVD	348
CUB2	528	ASRQGLT-VSFIPYFKEE-----	544
		*. * : . * . . .	

CUB2	417	-----CTDHRYCQRKSYSLQVPSDILHLPVELHDFSWKLLVPKDRLSLVLVPAQKLO	468
CUB3	545	GVFTVTPDTSKSVYLRTPNWDRG-----LPSLTSVSWNISVPRDQVACLTFKFKE---	593
		. : * : . . . * . * * : * * : * : : . . :	
CUB2	469	QHTHEKPCNTSFSYLVAS-----AIPSQDLYFG-----SFCPPGGSIKQI	507
CUB3	594	--RSGVVCQTGRAFMIIQEQRTRAEEIFSLDEDVLPKPSFHHSFWVNISNCSPTSGKQL	651
		* : * . : : : . . . * * * * * * :	
CUB2	508	QVKQNISVTLRTRFAPSFQQEASRQGLTVSFIPYFKEE	544
CUB3	652	DLLFS--VTLT-----	660
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CUB1	221	CIIESVFEGEGSATLMSANYPEGFPEDDELMTWQFVVPAPHLRASVSFLNFNLSNCERKEER	280
CUB3	545	GVFTVTPDTSKSVYLRTPNWDRGLPSLTSVSWNISVPRDQVACLTFKERSGVVCQTGRA	604
		: : . : : . . * : * : * * * . : * : * * . * : * : : . . : . .	
CUB1	281	VEYYIPGSTTNPEVFKLEDKQ---PGNMAGNFNLSLQGCQDQAQSPGILRLQFQVLVQHP	337
CUB3	605	FMIIQEQRTRAEEIFSLDEDVLPKPSFHHSFWVNISNCSPTSG--KQLDLLFSVTLT--	660
		. * * : * * : : . * . * . * : : . . * . : * * * * :	
CUB1	338	QNESNKIYVVD	348
CUB3		-----	