

From proteome to application by applied proteomics.  
Validation through a new antitumor compound.

**Authors:**

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**Supplementary Figure S1. Sequence alignment of the sequencing results.** CLUSTAL O(1.2.4) multiple sequence alignment of the sequencing results of the cloned prohibitin in pET28a. The sequencing results of the cloned prohibitin in pET28a were aligned with the nucleotide sequence XM\_005854224.1 (GenPept: XP\_005854286 /UniProt Accession: K8YWQ7) .

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	*****	
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**Supplementary Figure S2. Comparative sequence between EWM26807(W7TL69) and EKU22077(K8YWQ7). CLUSTAL O (1.2.4) multiple sequence alignment of EWM26807(W7TL69) and EKU22077(K8YWQ7) (A) nucleotides sequences; and (B) amino acid sequences.**

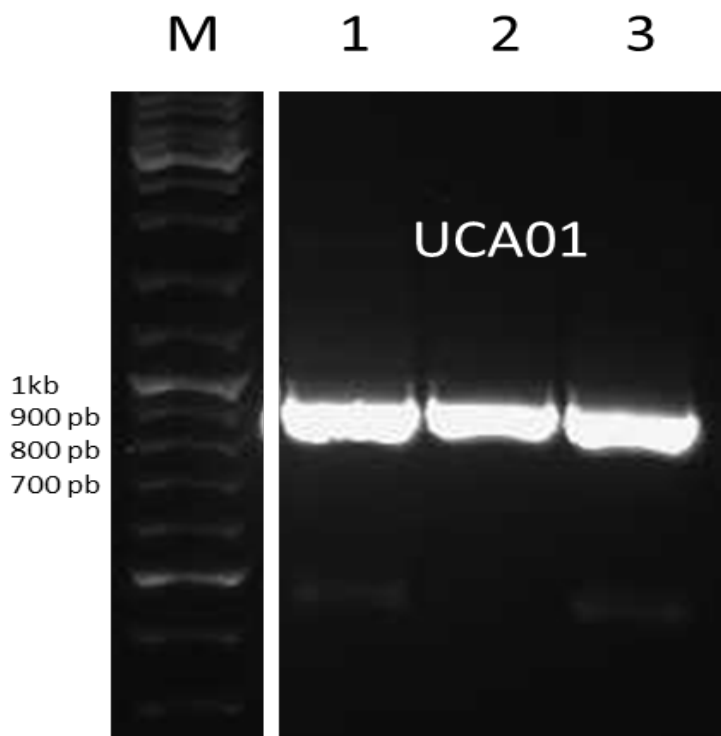
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ENA   EKU22077   EKU22077.1	-----ATGTCTCCAGCA	12
ENA   EWM26807   EWM26807.1	ATGGGGGTGGCGGGGTGGCGGGGGTGGAGGGGGTGGCCCGGGGCATGTCTCCAGCA *****	120
ENA   EKU22077   EKU22077.1	GGACCGCTGGGAGCCTTGTCTAGGTGTGACGGGCATCCTTTACGCGGGTACAATAGTTTT	72
ENA   EWM26807   EWM26807.1	GGACCGCTGGGAGCCTTGTCTAGGTGTGACGGGCATCCTTTACGCGGGTACAATAGTTTT *****	180
ENA   EKU22077   EKU22077.1	TACACGGTGGAGGGTGGCAACCGAGCTCTGTGTTCAACAGATTAATCGGTGTGAAAGAA	132
ENA   EWM26807   EWM26807.1	TACACGGTGGAGGGTGGCAACCGAGCTCTGTGTTCAACAGATTAATCGGTGTGAAAGAA *****	240
ENA   EKU22077   EKU22077.1	GAAGTGTACATGGAGGGAATGCATTTTATGATTCCCTGGTTCGACATGCCATCATTTAC	192
ENA   EWM26807   EWM26807.1	GAAGTGTACATGGAGGGAATGCATTTTATGATTCCCTGGTTCGACATGCCATCATTTAC *****	300
ENA   EKU22077   EKU22077.1	GACATCCGCCCAAGCCCGGATGATCCAGTCCCTTACAGGAAGCAAGACATGCAAATG	252
ENA   EWM26807   EWM26807.1	GACATCCGCCCAAGCCCGGATGATCCAGTCCCTTACAGGAAGCAAGACATGCAAATG *****	360
ENA   EKU22077   EKU22077.1	GTCAACATCACCATCCGCGTTTGTCTAAGCCCGACTCGGCTCAACTCCGCTGGATCTTC	312
ENA   EWM26807   EWM26807.1	GTCAACATCACCATCCGCGTTTGTCTAAGCCCGACTCGGCTCAACTCCGCTGGATCTTC *****	420
ENA   EKU22077   EKU22077.1	CGCACCTTGGGTCGCGACTACGACGAGCGTGTCTCCCTCCATCGTCAACGAGGTCCTCC	372
ENA   EWM26807   EWM26807.1	CGCACCTTGGGTCGCGACTACGACGAGCGTGTCTCCCTCCATCGTCAACGAGGTCCTCC *****	480
ENA   EKU22077   EKU22077.1	AAGGCCGTGGTGGCCAAGTACAACGCCCGGAGCTCTTGACGAAGCGTGAGATGGTCTCC	432
ENA   EWM26807   EWM26807.1	AAGGCCGTGGTGGCCAAGTACAACGCCCGGAGCTCTTGACGAAGCGTGAGATGGTCTCC *****	540
ENA   EKU22077   EKU22077.1	ACCCAAATCCGGTTGCGAGTTGGAGAAGCGTGGAAAGGAGTTTCGGATCGTCCGGACGAC	492
ENA   EWM26807   EWM26807.1	ACCCAAATCCGGTTGCGAGTTGGAGAAGCGTGGAAAGGAGTTTCGGATCGTCCGGACGAC *****	600
ENA   EKU22077   EKU22077.1	GTGTGATCACCCACTTACCTTCTCCCGGGAGTACACGAACCGGTCGAGGCCAAGCAA	552
ENA   EWM26807   EWM26807.1	GTGTGATCACCCACTTACCTTCTCCCGGGAGTACACGAACCGGTCGAGGCCAAGCAA *****	660
ENA   EKU22077   EKU22077.1	GTGTCTCAACAGGAAGCCGAGCGCGCAAATATGTGGTAATGAAAGCGAACCAAGAAAAG	612
ENA   EWM26807   EWM26807.1	GTGTCTCAACAGGAAGCCGAGCGCGCAAATATGTGGTAATGAAAGCGAACCAAGAAAAG *****	720
ENA   EKU22077   EKU22077.1	GAAGCCATCATATCAAAGCGGAGGGAGAGGCCAATCCGCTGCTCTGGTGGTAAAGGCC	672
ENA   EWM26807   EWM26807.1	GAAGCCATCATATCAAAGCGGAGGGAGAGGCCAATCCGCTGCTCTGGTGGTAAAGGCC *****	780
ENA   EKU22077   EKU22077.1	ATTCGAGAGAATCCTGCTTTTATCAAGCTGCGCAAAATCGACGCGACGAGGACATTGCG	732
ENA   EWM26807   EWM26807.1	ATTCGAGAGAATCCTGCTTTTATCAAGCTGCGCAAAATCGACGCGACGAGGACATTGCG *****	840
ENA   EKU22077   EKU22077.1	AATGTCGTGCTCTCTCGGGTCAGAAAGTCTATCTGTCTCGGACTCCCTCTTGTGAAT	792
ENA   EWM26807   EWM26807.1	AATGTCGTGCTCTCTCGGGTCAGAAAGTCTATCTGTCTCGGACTCCCTCTTGTGAAT *****	900
ENA   EKU22077   EKU22077.1	ATGTACTCGGGCGACGAAGAGAAGTCTGGAAGAAGCGGTAG	834
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	*****	

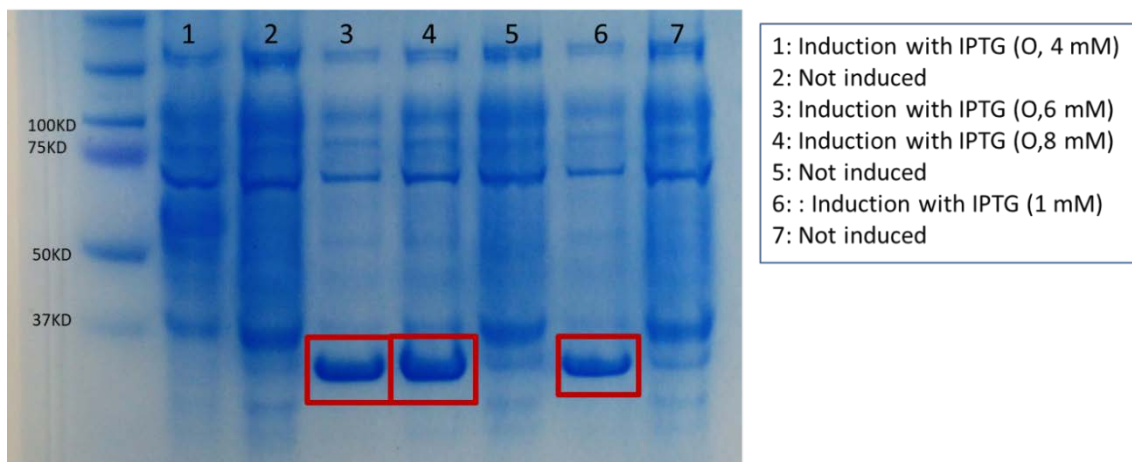
**B.**

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tr   W7TL69   W7TL69_9STRA	MNAARKFAENLQSKI PKGAGMGGGAGGGGGGPGGMS PAGPLGALLGVTGILYAGYNSF *****	60
tr   K8YWQ7   K8YWQ7_NANGC	YTVEGGHRALLFNRLIGVKEEVYMEGMHFMIPWFDMPIIYDIRPKPRMIQSLTGSKDMQM	84
tr   W7TL69   W7TL69_9STRA	YTVEGGHRALLFNRLIGVKEEVYMEGMHFMIPWFDMPIIYDIRPKPRMIQSLTGSKDMQM *****	120
tr   K8YWQ7   K8YWQ7_NANGC	VNITIRVLSKPDQAQLRWIFRILGRDYDERVLPDIVNEVSKAVVAKYNAEELLTKREMSV	144
tr   W7TL69   W7TL69_9STRA	VNITIRVLSKPDQAQLRWIFRILGRDYDERVLPDIVNEVSKAVVAKYNAEELLTKREMSV *****	180
tr   K8YWQ7   K8YWQ7_NANGC	TQIRLQLEKRAKEFRIVLDDVSI THLTF SREYTNAVEAKQVAQEAERAKYVVMKANQEK	204
tr   W7TL69   W7TL69_9STRA	TQIRLQLEKRAKEFRIVLDDVSI THLTF SREYTNAVEAKQVAQEAERAKYVVMKANQEK *****	240
tr   K8YWQ7   K8YWQ7_NANGC	EAI I IKAEGEAQSAALVGKAIARENPAFIKLRKIDAARDIANVVS SSGQKVVLSADSLLLN	264
tr   W7TL69   W7TL69_9STRA	EAI I IKAEGEAQSAALVGKAIARENPAFIKLRKIDAARDIANVVS SSGQKVVLSADSLLLN *****	300
tr   K8YWQ7   K8YWQ7_NANGC	MYSGDEEKSGKKR	277
tr   W7TL69   W7TL69_9STRA	MYSGDEEKSGKKR	313
	*****	

**Supplementary Figure S3. Amplification of UCA01 DNA.** Fragment of Prohibitin (834 bp) amplified by RT-PCR from *N. gaditana* RNA. Lane M: Molecular weight ladder; Lane 1, 2 and 3: Prohibitin fragment (NCBI Accession: XM\_005854224.1/UniProt Accession: K8YWQ7).



**Supplementary Figure S4. Expression of UCA01 in Rosetta gami.** 10% SDS-PAGE gel of *N. gaditana* UCA01 heterologous expression by *E. coli* Rosetta gami. Transformants of *E. coli* Rosetta gami with pET28a carrying *N. gaditana* prohibitin gene was cultured at 37°C for 2.5-3 hours in LB with (Induction of plasmid expression) or without IPTG (No induction of plasmid expression). After mechanical lysis of the cultures, the soluble fraction was analysed by SDS-PAGE. Lane M: Molecular weight ladder. Lane 1: Induction of recombinant protein expression with 1mM of IPTG. Lane 2: No induction of recombinant protein expression (without IPTG). SDS-PAGE gel was run at 200V for 45 min and stained with Coomassie blue.



**Supplementary Figure S5. UCA01 Purification.** 10% SDS-PAGE gel of *N. gaditana* recombinant UCA01 purification. Induced culture of *E. coli* Rosseta gami expressing *N. gaditana* prohibitin gene was lysated and its soluble fraction was purified by metal ion affinity chromatography. The steps of the purification process were analysed by SDS-PAGE. Lane M: Molecular weight ladder. Lane A: No induced culture of transformant *E. coli* Rosseta gami (not purified fraction). Lane B: Induced culture of transformant *E. coli* Rosseta gami before purification. Lane C: load step of purification process using induced culture of transformant *E. coli* Rosseta gami. Lane D: Wash step of purification process using induced culture of transformant *E. coli* Rosseta gami. Lane E: First elution step of purification process using induced culture of transformant *E. coli* Rosseta gami. Lane F: Second elution step of purification process using induced culture of transformant *E. coli* Rosseta gami. SDS-PAGE gel was run at 200V for 45 min and stained with Coomassie blue.

