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

Authors:

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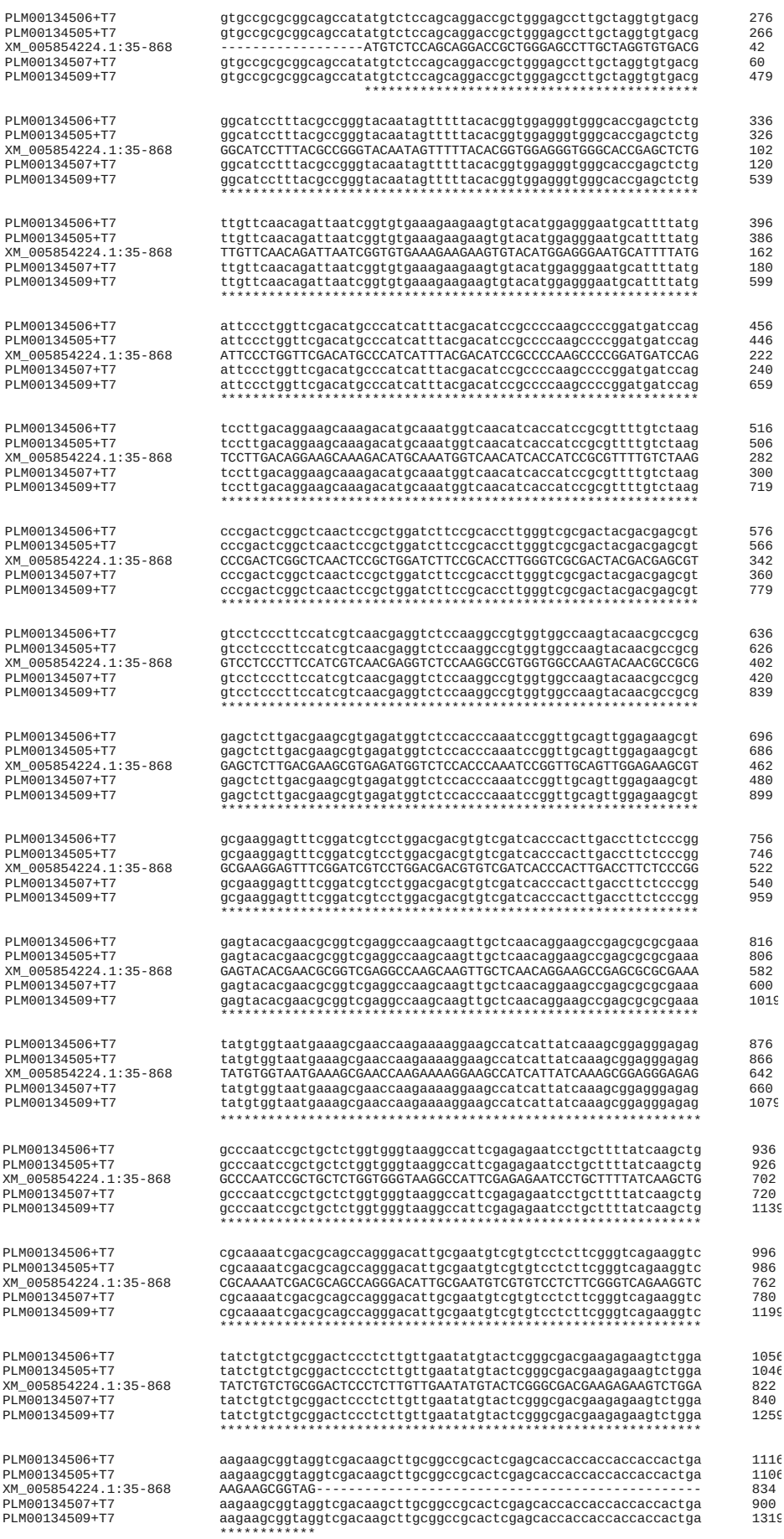
Supplementary Figure S1. Sequence alignment of the sequencing results. CLUSTAL O(1.2.4) multiple sequence alignment of the sequencing results of the cloned prohibitin in pET28a. The sequencing results of the cloned prohibitin in pET28a were aligned with the nucleotide sequence XM_005854224.1 (GenPept: XP_005854286 /UniProt Accession: K8YWQ7) .



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.

A.

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ENA|EKU22077|EKU22077.1       ------------------------------------------------------------    0
ENA|EWM26807|EWM26807.1       ATGAATGCAGCCAAGAAGTTTGCCGAGAACCTGGGTAGCAAAATTCCCAAGGGTGCTGGC    60

ENA|EKU22077|EKU22077.1       ---------------------------------------------ATGTCTCCAGCA    12
ENA|EWM26807|EWM26807.1       ATGGGGGGTGGCGCGGGTGGCGGGGGTGGAGGGGGTGGCCCCGGGGGCATGTCTCCAGCA    120
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ENA|EKU22077|EKU22077.1       GGACCGCTGGGAGCCTTGCTAGGTGTGACGGGCATCCTTTACGCCGGGTACAATAGTTTT    72
ENA|EWM26807|EWM26807.1       GGACCGCTGGGAGCCTTGCTAGGTGTGACGGGCATCCTTTACGCCGGGTACAATAGTTTT    180
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ENA|EKU22077|EKU22077.1       TACACGGTGGAGGGTGGGCACCGAGCTCTGTTGTTCAACAGATTAATCGGTGTGAAAGAA    132
ENA|EWM26807|EWM26807.1       TACACGGTGGAGGGTGGGCACCGAGCTCTGTTGTTCAACAGATTAATCGGTGTGAAAGAA    240
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ENA|EKU22077|EKU22077.1       GAAGTGTACATGGAGGGAATGCATTTTATGATTCCCTGGTTCGACATGCCCATCATTTAC    192
ENA|EWM26807|EWM26807.1       GAAGTGTACATGGAGGGAATGCATTTTATGATTCCCTGGTTCGACATGCCCATCATTTAC    300
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ENA|EKU22077|EKU22077.1       GACATCCGCCCCAAGCCCCGGATGATCCAGTCCTTGACAGGAAGCAAAGACATGCAAATG    252
ENA|EWM26807|EWM26807.1       GACATCCGCCCCAAGCCCCGGATGATCCAGTCCTTGACAGGAAGCAAAGACATGCAAATG    360
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ENA|EKU22077|EKU22077.1       GTCAACATCACCATCCGCGTTTTGTCTAAGCCCGACTCGGCTCAACTCCGCTGGATCTTC    312
ENA|EWM26807|EWM26807.1       GTCAACATCACCATCCGCGTTTTGTCTAAGCCCGACTCGGCTCAACTCCGCTGGATCTTC    420
                              ************************************************************

ENA|EKU22077|EKU22077.1       CGCACCTTGGGTCGCGACTACGACGAGCGTGTCCTCCCTTCCATCGTCAACGAGGTCTCC    372
ENA|EWM26807|EWM26807.1       CGCACCTTGGGTCGCGACTACGACGAGCGTGTCCTCCCTTCCATCGTCAACGAGGTCTCC    480
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ENA|EKU22077|EKU22077.1       AAGGCCGTGGTGGCCAAGTACAACGCCGCGGAGCTCTTGACGAAGCGTGAGATGGTCTCC    432
ENA|EWM26807|EWM26807.1       AAGGCCGTGGTGGCCAAGTACAACGCCGCGGAGCTCTTGACGAAGCGTGAGATGGTCTCC    540
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ENA|EKU22077|EKU22077.1       ACCCAAATCCGGTTGCAGTTGGAGAAGCGTGCGAAGGAGTTTCGGATCGTCCTGGACGAC    492
ENA|EWM26807|EWM26807.1       ACCCAAATCCGGTTGCAGTTGGAGAAGCGTGCGAAGGAGTTTCGGATCGTCCTGGACGAC    600
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ENA|EKU22077|EKU22077.1       GTGTCGATCACCCACTTGACCTTCTCCCGGGAGTACACGAACGCGGTCGAGGCCAAGCAA    552
ENA|EWM26807|EWM26807.1       GTGTCGATCACCCACTTGACCTTCTCCCGGGAGTACACGAACGCGGTCGAGGCCAAGCAA    660
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ENA|EKU22077|EKU22077.1       GTTGCTCAACAGGAAGCCGAGCGCGCGAAATATGTGGTAATGAAAGCGAACCAAGAAAAG    612
ENA|EWM26807|EWM26807.1       GTTGCTCAACAGGAAGCCGAGCGCGCGAAATATGTGGTAATGAAAGCGAACCAAGAAAAG    720
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ENA|EKU22077|EKU22077.1       GAAGCCATCATTATCAAAGCGGAGGGAGAGGCCCAATCCGCTGCTCTGGTGGGTAAGGCC    672
ENA|EWM26807|EWM26807.1       GAAGCCATCATTATCAAAGCGGAGGGAGAGGCCCAATCCGCTGCTCTGGTGGGTAAGGCC    780
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ENA|EKU22077|EKU22077.1       ATTCGAGAGAATCCTGCTTTTATCAAGCTGCGCAAAATCGACGCAGCCAGGGACATTGCG    732
ENA|EWM26807|EWM26807.1       ATTCGAGAGAATCCTGCTTTTATCAAGCTGCGCAAAATCGACGCAGCCAGGGACATTGCG    840
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ENA|EKU22077|EKU22077.1       AATGTCGTGTCCTCTTCGGGTCAGAAGGTCTATCTGTCTGCGGACTCCCTCTTGTTGAAT    792
ENA|EWM26807|EWM26807.1       AATGTCGTGTCCTCTTCGGGTCAGAAGGTCTATCTGTCTGCGGACTCCCTCTTGTTGAAT    900
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ENA|EKU22077|EKU22077.1       ATGTACTCGGGCGACGAAGAGAAGTCTGGAAAGAAGCGGTAG      834
ENA|EWM26807|EWM26807.1       ATGTACTCGGGCGACGAAGAGAAGTCTGGAAAGAAGCGGTAG      942
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B.

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tr|K8YWQ7|K8YWQ7_NANGC        ----------------------------------MSPAGPLGALLGVTGILYAGYNSF     24
tr|W7TL69|W7TL69_9STRA        MNAAKKFAENLGSKIPKGAGMGGGAGGGGGGGGPGGMSPAGPLGALLGVTGILYAGYNSF     60
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tr|K8YWQ7|K8YWQ7_NANGC        YTVEGGHRALLFNRLIGVKEEVYMEGMHFMIPWFDMPIIYDIRPKPRMIQSLTGSKDMQM     84
tr|W7TL69|W7TL69_9STRA        YTVEGGHRALLFNRLIGVKEEVYMEGMHFMIPWFDMPIIYDIRPKPRMIQSLTGSKDMQM     120
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tr|K8YWQ7|K8YWQ7_NANGC        VNITIRVLSKPDSAQLRWIFRTLGRDYDERVLPSIVNEVSKAVVAKYNAAELLTKREMVS     144
tr|W7TL69|W7TL69_9STRA        VNITIRVLSKPDSAQLRWIFRTLGRDYDERVLPSIVNEVSKAVVAKYNAAELLTKREMVS     180
                              ************************************************************

tr|K8YWQ7|K8YWQ7_NANGC        TQIRLQLEKRAKEFRIVLDDVSITHLTFSREYTNAVEAKQVAQQEAERAKYVVMKANQEK     204
tr|W7TL69|W7TL69_9STRA        TQIRLQLEKRAKEFRIVLDDVSITHLTFSREYTNAVEAKQVAQQEAERAKYVVMKANQEK     240
                              ************************************************************

tr|K8YWQ7|K8YWQ7_NANGC        EAIIIKAEGEAQSAALVGKAIRENPAFIKLRKIDAARDIANVVSSSGQKVYLSADSLLLN     264
tr|W7TL69|W7TL69_9STRA        EAIIIKAEGEAQSAALVGKAIRENPAFIKLRKIDAARDIANVVSSSGQKVYLSADSLLLN     300
                              ************************************************************

tr|K8YWQ7|K8YWQ7_NANGC        MYSGDEEKSGKKR  277
tr|W7TL69|W7TL69_9STRA        MYSGDEEKSGKKR  313
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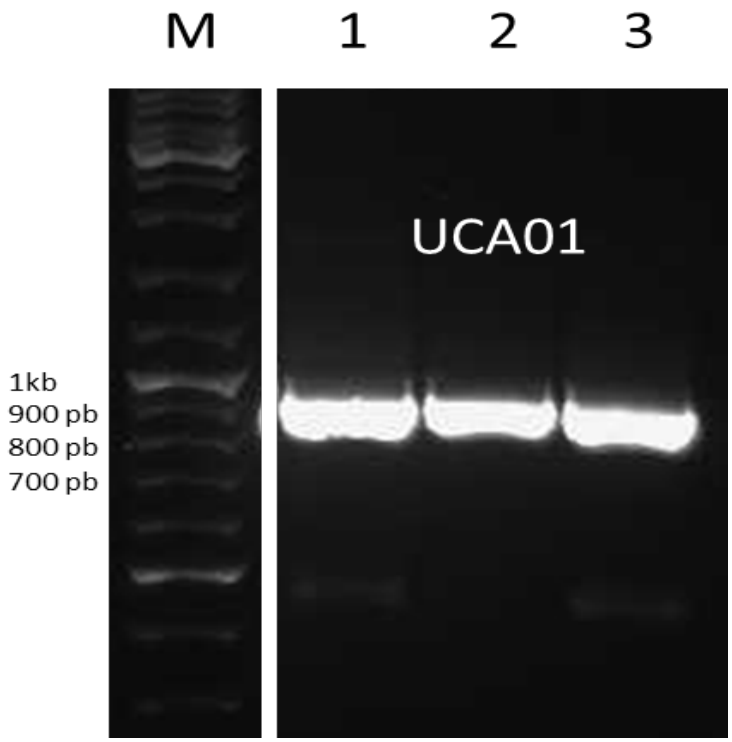
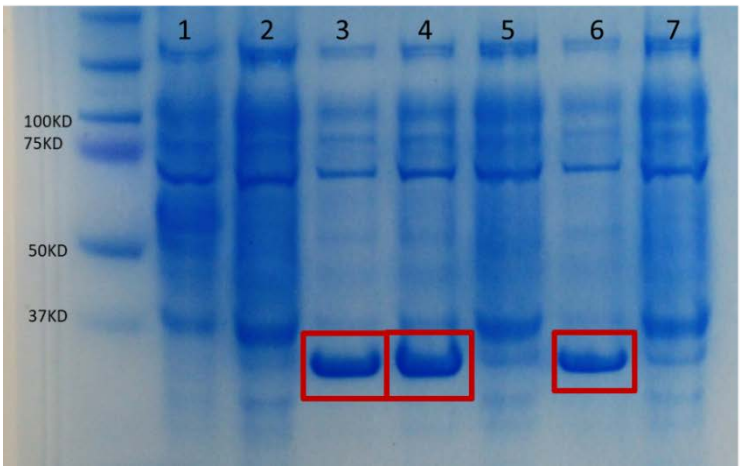
Supplementary Figure S3. **Amplification of UCA01 DNA.** Fragment of Prohibitin (834 bp) amplified by RT-PCR from *N. gaditana* RNA. Lane M: Molecular weight ladder; Lane 1, 2 and 3: Prohibitin fragment (NCBI Accession: XM_005854224.1/UniProt Accession: K8YWQ7).



Supplementary Figure S4. **Expression of UCA01 in Rosetta gami**. 10% SDS-PAGE gel of *N. gaditana* UCA01 heterologous expression by *E. coli* Rosseta gami. Transformants of *E. coli* Rosseta gami with pET28a carrying *N. gaditana* prohibitin gene was cultured at 37ºC 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.