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From Sea to Therapy: Development and analytical control of recombinant human CDKL5 production in the marine bacterium P*seudoalteromonas haloplanktis* TAC125

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Supplementary

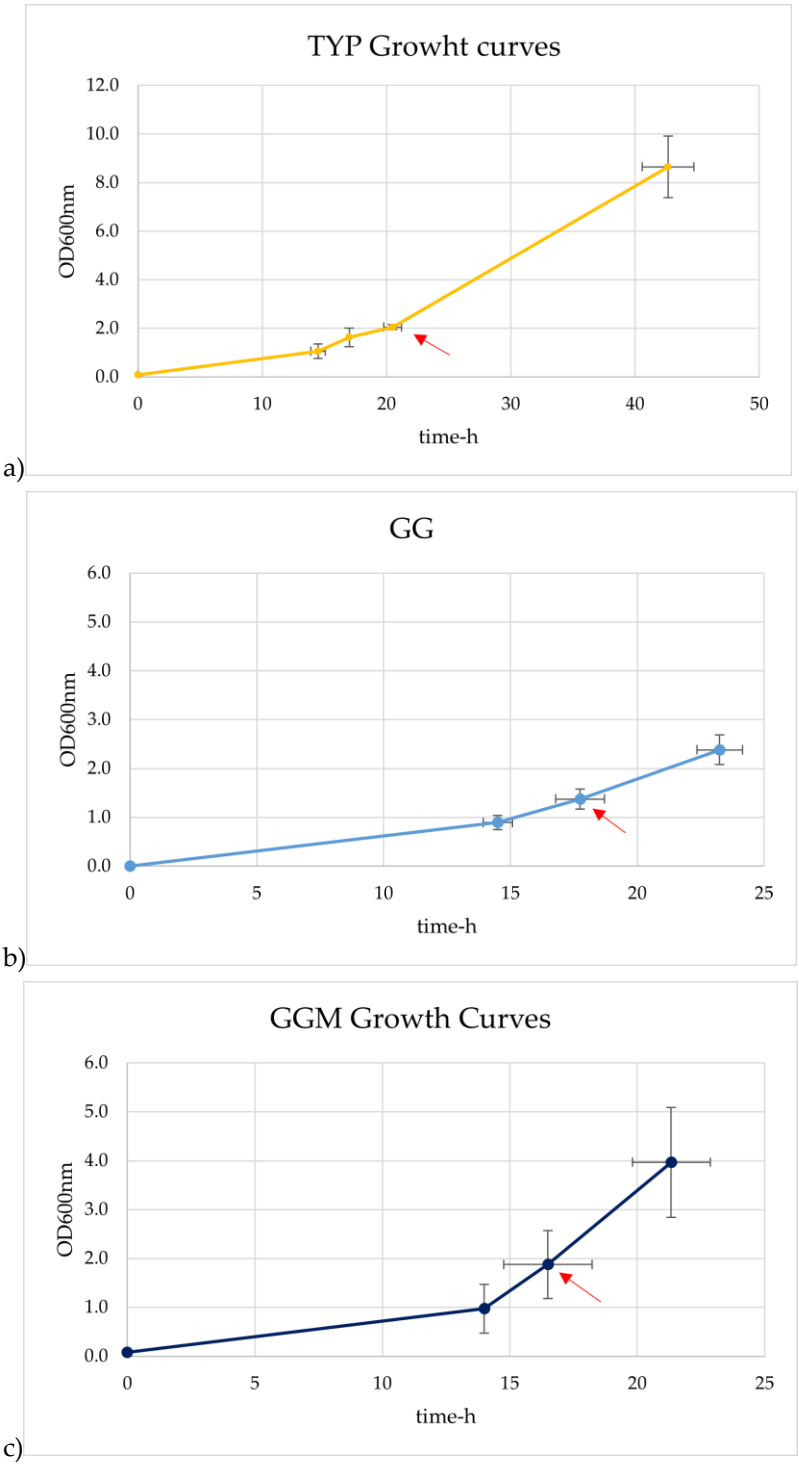


Figure S1. *Ph*TAC125 KrPl *LacY*$^{+}$ pB40-BDC2-CDKL5(G7opt)[1–4]. Growth curves in TYP(a), GG (b) and GGM media [5–7]. Cultures in the three media were grown at 15 °C with agitation at 220 rpm in 250 mL Erlenmeyer flasks [4]. Recombinant production was induced with 5 mM IPTG (red arrows). Cellular concentration was monitored by measuring absorbance at 600nm using a spectrophotometer. The growth curves are the average obtained from three independent replicas.

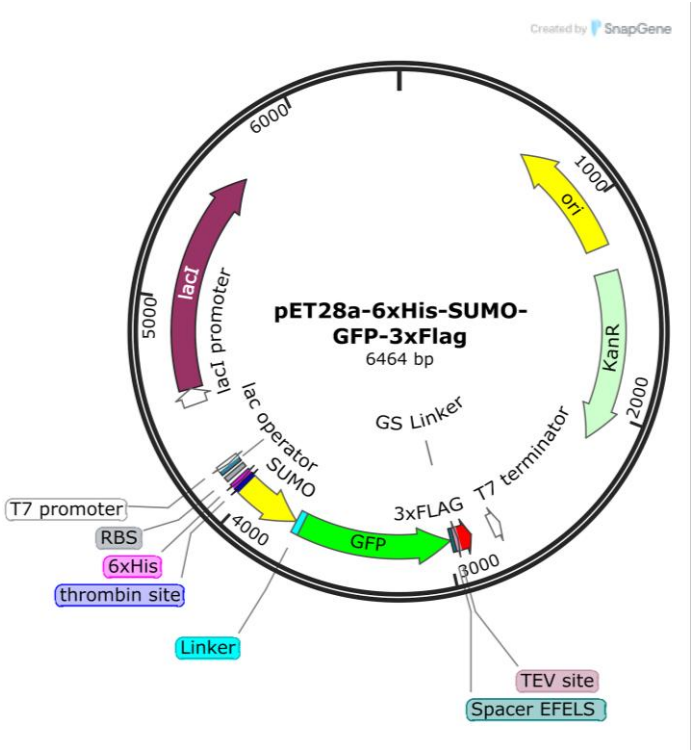


Figure S2. ELISA Standard 6xHis-SUMO-GFP-3xFLAG expression vector map. The *egfp* sequence was cloned inside the vector pET28a-6xHis-SUMO-3xFlag, between the SUMO-tag and the 3xFLAG-tag by Gibson assembly method [8,9]. The map illustrates the genetic structure of the plasmid backbone: ori: origin of replication in *E. coli* strain; KanR: Kanamycin resistance gene; 6xHis-SUMO-GFP-3xFLAG construct; RBS. ribosome binding site; T7 promoter sequence; LacI gene. FASTA sequence in Supplementary2_pET28a-6xHis-SUMO-3xFLAG file.

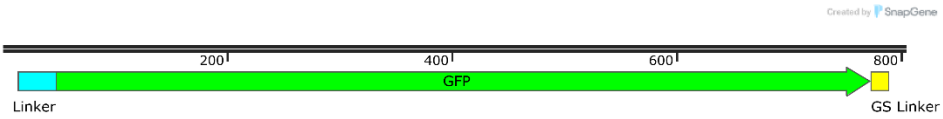


Figure S3. *egfp gene* insert sequence. The picture shows the *egfp* gene fragment inserted into the receiving vector (pET28a-6xHis-SUMO-3xFLAG) via Gibson assembly method for the construction of the ELISA standard protein [9]. The *egfp gene* insert fragment was amplified by PCR using Q5 polymerase and the primers *egfp*_fwd and *egfp*_rev. Template sequence reported in the Supplementary material as a FASTA file (*Supplementary_egfp_gene_insert*). In light blue, the linker (Sequence reported in Table S.2) for was localised at 5′of the sequence; meanwhile, a GS linker was added at the 3′ . FASTA sequence in Supplementary3_egfp_gene insert file.

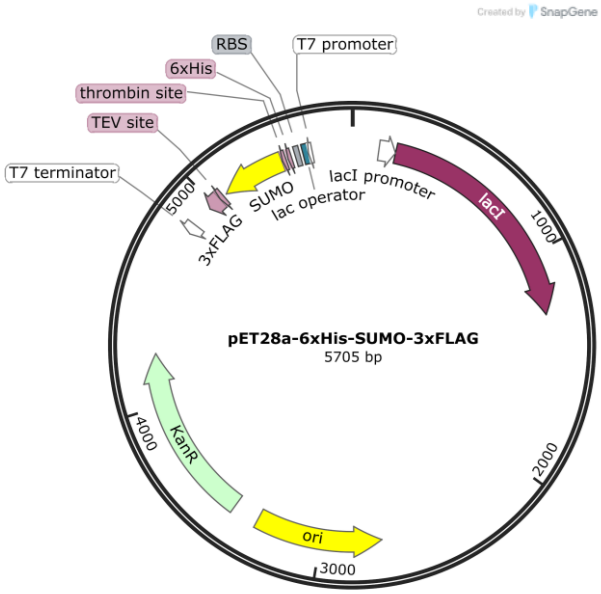


Figure S.4 Standard 6xHis-SUMO-3xFLAG expression vector map used as receiving vector for the Gibson assembly [10]. The receiving vector was amplified by PCR using the primers pET28a-SUMO-3xFLAG_fwd and pET28a-SUMO-3xFLAG_rev.The map illustrates the genetic structure of the plasmid backbone: ori: origin of replication in *E. coli* host; KanR: Kanamycin resistance gene; 6xHis-SUMO-3xFLAG construct; RBS: ribosome binding site; T7 promoter sequence; LacI gene. FASTA sequence in Supplementary4_pET28a-6xHis-SUMO-3xFLAG file.

Table S.1 Gibson Assembly Primers used for the cloning of the pET28a-6xHis-SUMO-3xFLAG vector[10].

Primers	**Sequence**
*egfp*_fwd	GTGGATCCCCAGCCGGGGCTCTGATTAA
*egfp*_rev	CTCGAATTCTTTGTAGAGCTCATCCATGCCATGTG
pET28a-SUMO-3xFLAG_fwd	CGGCTGGGGATCCACCAATCTGTTCACG
pET28a-SUMO-3xFLAG_rev	GCTCTACAAAGAATTCGAGCTCTCTGAGAATTTATA TTTCCAAGGTG

Primers were designed to clone the *egfp* sequence into the pET28a-6xHis-SUMO-3xFLAG vector via Gibson Assembly.

Table S.2 6xHis-SUMO-eGFP-3xFLAG and recombinant hCDKL5 amino acid sequence.

Protein	Amino acid sequence
6xHis-SUMO-eGFP-3xFLAG 49kDa	MGSSHHHHHHSSGLVPRGSHMGSDSEVNQEAKPEVKPEVKPETHIN LKVSDGSSEIFFKIKKTTPLRRLMEAFAKRQGKEMDSLRFLYDGIRIQA DQTPEDLDMEDNDIIEAHREQIGGSPAGALINMHGTGGGNSSKGEE LFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPV PWPTLVTTLTYGVQCFSRYPDHMKRHDFFKSAMPEGYVQERTISFKD DGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNV YITADKQKNGIKANFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDN HYLSTQSALSKDPNEKRDHMVLLEFVTAAGITHGMDELYKEFELSENL YFQGDYKDHDGDYKDHDIDYKDDDDK	
Recombinant hCDKL5 129kDa	MASAWSHPQFEKGGGSGGGSGGSAWSHPQFEKGSDSEVNQEAKP EVKPEVKPETHINLKVSDGSSEIFFKIKKTTPLRRLMEAFAKRQGKEMD SLRFLYDGIRIQADQTPEDLDMEDNDIIEAHREQIGGMGDAAQPARR ARRTKLAAYARKAARQARAGGGGSKIPNIGNVMNKFEILGVVGEGAY GVVLKCRHKETHEIVAIKKFKDSEENEEVKETTLRELKMLRTLKQENIVE LKEAFRRRGKLYLVFEYVEKNMLELLEEMPNGVPPEKVKSYIYQLIKAI HWCHKNDIVHRDIKPENLLISHNDVLKLCDFGFARNLSEGNNANYTE YVATRWYRSPELLLGAPYGKSVDMWSVGCILGELSDGQPLFPGESEID QLFTIQKVLGPLPSEQMKLFYSNPRFHGLRFPAVNHPQSLERRYLGILN SVLLDLMKNLLKLDPADRYLTEQCLNHPTFQTQRLLDRSPSRSAKRKP YHVESSTLSNRNQAGKSTALQSHHRSNSKDIQNLSVGLPRADEGLPA NESFLNGNLAGASLSPLHTKTYQASSQPGSTSKDLTNNNIPHLLSPKEA KSKTEFDFNIDPKPSEGPGTKYLKSNSRSQQNRHSFMESSQSKAGTLQ PNEKQSRHSYIDTIPQSSRSPSYRTKAKSHGALSDSKSVSNLSEARAQIA EPSTSRYFPSSCLDLNSPTSPTPTRHSDTRTLLSPSGRNNRNEGTLDSR RTTTRHSKTMEELKLPEHMDSSHSHSLSAPHESFSYGLGYTSPFSSQQ RPHRHSMYVTRDKVRAKGLDGSLSIGQGMAARANSLQLLSPQPGEQ LPPEMTVARSSVKETSREGTSSFHTRQKSEGGVYHDPHSDDGTAPKE NRHLYNDPVPRRVGSFYRVPSPRPDNSFHENNVSTRVSSLPSESSSGT NHSKRQPAFDPWKSPENISHSEQLKEKEKQGFFRSMKKKKKKSQTVP NSDSPDLLTLQKSIHSASTPSSRPKEWRPEKISDLQTQSQPLKSLRKLLH LSSASNHPASSDPRFQPLTAQQTKNSFSEIRIHPLSQASGGSSNIRQEP APKGRPALQLPGQMDPGWHVSSVTRSATEGPSYSEQLGAKSGPNG HPYNRTNRSRMPNLNDLKETALGGGGSENLYFQGDYKDHDGDYKD HDIDYKDDDDK	

The full amino acid sequence of the Standard protein and hCDKL5 are displayed. For 6xHis-SUMO-eGFP-3xFLAG, the following schematisation is used: 6xHis-tag is represented in dark grey, thrombine binding site in pink, SUMO-tag sequence in light blue, eGFP protein sequence in yellow, TEV protease binding site in purple and 3xFLAG-tag in red. For the hCDKL5 protein sequence, the following schematisation is used: TwinStrep-Tag is represented in light grey, SUMO-tag sequence in light blue, TATk signal peptide sequence is dark blue [11], recombinant hCDKL5 sequence is represented in orange (Catalytic domain is represented in orange. IDR domain is represented in orange), TEV protease binding site in purple and 3xFLAG-tag in red. All speaker sequences are left in black.

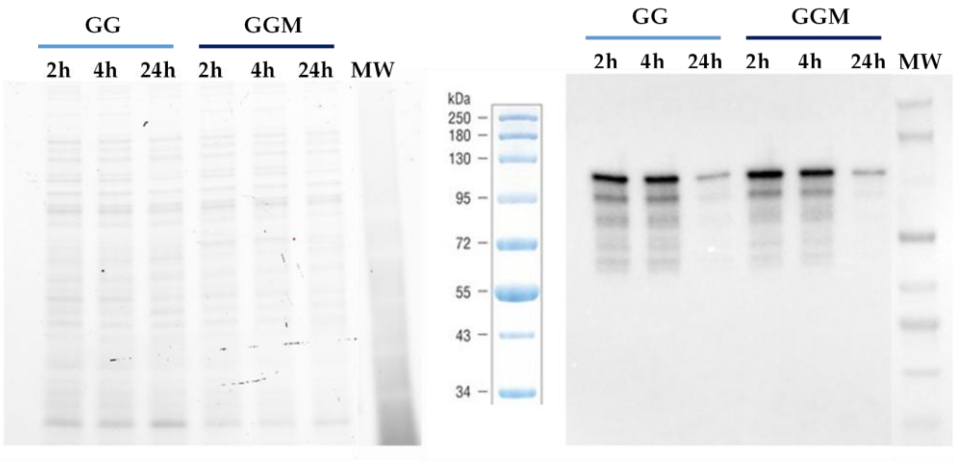


Figure S.5 Solubility of hCDKL5 produced in *Ph*TAC125 with GG and GGM media [1,2,4,5]. On the left the SDS-page coomassie stained, on the right Western blot analysis performed with the anti-CDKL5 antibody. The samples were collected 2, 4 and 24 hours after the induction with 5mM IPTG. MW: Molecular weight.

References

1. Colarusso, A.; Lauro, C.; Calvanese, M.; Parrilli, E.; Tutino, M.L. Active Human Full-Length CDKL5 Produced in the Antarctic Bacterium Pseudoalteromonas Haloplanktis TAC125. *Microb. Cell Fact.* **2022**, *21*, doi:10.1186/s12934-022-01939-6.
2. Colarusso, A.; Lauro, C.; Calvanese, M.; Parrilli, E.; Tutino, M.L. Improvement of Pseudoalteromonas Haloplanktis Tac125 as a Cell Factory: Iptg-Inducible Plasmid Construction and Strain Engineering. *Microorganisms* **2020**, *8*, 1–24, doi:10.3390/microorganisms8101466.
3. Colarusso, A.; Lauro, C.; Canè, L.; Cozzolino, F.; Tutino, M.L. Bacterial Production of CDKL5 Catalytic Domain: Insights into Aggregation, Internal Translation and Phosphorylation Patterns. *Int. J. Mol. Sci.* **2024**, *25*, doi:10.3390/ijms25168891.
4. Calvanese, M.; Colarusso, A.; Lauro, C.; Parrilli, E.; Tutino, M.L. Soluble Recombinant Protein Production in Pseudoalteromonas Haloplanktis TAC125: The Case Study of the Full-Length Human CDKL5 Protein. In *Methods in Molecular Biology*; Humana Press Inc., Totowa, NJ, USA 2022; Vol. 2406, pp. 219–232.
5. Fondi, M.; Gonzi, S.; Dziurzynski, M.; Turano, P.; Ghini, V.; Calvanese, M.; Colarusso, A.; Lauro, C.; Parrilli, E.; Tutino, M.L. Modelling HCDKL5 Heterologous Expression in Bacteria. *Metabolites* **2021**, *11*, doi:10.3390/metabo11080491.
6. Sannino, F.; Giuliani, M.; Salvatore, U.; Apuzzo, G.A.; de Pascale, D.; Fani, R.; Fondi, M.; Marino, G.; Tutino, M.L.; Parrilli, E. A Novel Synthetic Medium and Expression System for Subzero Growth and Recombinant Protein Production in Pseudoalteromonas Haloplanktis TAC125. *Appl. Microbiol. Biotechnol.* **2017**, *101*, 725–734, doi:10.1007/s00253-016-7942-5.
7. Wilmes, B.; Hartung, A.; Lalk, M.; Liebeke, M.; Schweder, T.; Neubauer, P. *Fed-Batch Process for the Psychrotolerant Marine Bacterium Pseudoalteromonas Haloplanktis*; *Microb. Cell Factories* **2010**, *9*, 72.
8. Wild David *The Immunoassay Handbook Theory and Applications of Ligand Binding, ELISA and Related Techniques*; Fourth Edition.; 2013;
9. March, J.C.; Rao, G.; Bentley, W.E. Biotechnological Applications of Green Fluorescent Protein. *Appl. Microbiol. Biotechnol.* 2003, *62*, 303–315.

10. Gibson, D.G.; Young, L.; Chuang, R.Y.; Venter, J.C.; Hutchison, C.A.; Smith, H.O. Enzymatic Assembly of DNA Molecules up to Several Hundred Kilobases. *Nat. Methods* **2009**, *6*, 343–345, doi:10.1038/nmeth.1318.
11. Trazzi, S.; De Franceschi, M.; Fuchs, C.; Bastianini, S.; Viggiano, R.; Lupori, L.; Mazziotti, R.; Medici, G.; Martire, V. Lo; Ren, E.; et al. CDKL5 Protein Substitution Therapy Rescues Neurological Phenotypes of a Mouse Model of CDKL5 Disorder. *Hum. Mol. Genet.* **2018**, *27*, 1572–1592, doi:10.1093/hmg/ddy064.

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