## **Supplementary Information**

## Chp1 is a dedicated chaperone at the ribosome that safeguards eEF1A biogenesis

Melania Minoia<sup>1†</sup>, Jany Quintana-Cordero<sup>1†</sup>, Katharina Jetzinger<sup>1,2</sup>, Ilgin Eser Kotan<sup>2</sup>, Kathryn Jane Turnbull<sup>3,4</sup>, Michela Ciccarelli<sup>1</sup>, Anna E. Masser<sup>1</sup>, Dorina Liebers<sup>2</sup>, Eloïse Gouarin<sup>1</sup>, Marius Czech<sup>1</sup>, Vasili Hauryliuk<sup>5,6</sup>, Bernd Bukau<sup>2</sup>, Günter Kramer<sup>2</sup>, Claes Andréasson<sup>1\*</sup>

<sup>1</sup>Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm University, Stockholm, Sweden

<sup>2</sup>Center for Molecular Biology of the University of Heidelberg (ZMBH), DKFZ-ZMBH Alliance, Heidelberg, Germany

<sup>3</sup> Department of Clinical Microbiology, Rigshospitalet, 2200 Copenhagen, Denmark

<sup>4</sup>Department of Molecular Biology, Laboratory for Molecular Infection Medicine Sweden, Umeå Centre for Microbial Research, Science for Life Laboratory, Umeå University, Umeå, Sweden

<sup>5</sup> Science for Life Laboratory, Department of Experimental Medical Science, Lund University, Lund, Sweden.

<sup>6</sup>University of Tartu, Institute of Technology, 50411 Tartu, Estonia

†Contributed equally

\*Corresponding author. Email: claes.andreasson@su.se

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Supplementary Fig. 1: Chp1 is a highly conserved protein that forms a complex with NAC and RNC complexes. a Overlap of 3D-structures of yeast Chp1 in blue (PDB ID. 2JYN) and the ColabFold model of the human ortholog PBDC1 (Uniprot accession: Q08971, amino acids 13-172,) in green. b Photo-crosslinking (+UV) of yeast cells grown at 30 °C expressing Chp1-HA with Bpa (+Bpa) incorporated at amino acid positions 12, 15, 120, 122, 83 or 89. Proteins were visualized by western blot analysis. Experiment was performed three times. c Photo-crosslinking (+UV) of yeast cells grown at 30 °C expressing Chp1-HA-myc-8xHis with Bpa incorporated at amino acid positions 12 or 122. Proteins were visualized by western blot analysis. Experiment was performed three times. d Ribbon diagram of ColabFold models showing the predicted complex between Chp1 and NAC from yeast and humans. Chp1 and PBDC1 in blue, βNAC in violet (amino acids 32-191 of the human protein are shown) and  $\alpha$ NAC in red (amino acids 64-215 of the human protein are shown). The UBA domain of  $\alpha$ NAC in the yeast and human proteins is marked. Note that the heterodimerization domain structurally determined by crystallography is arranged in a head-to-head manner that generates a 12-stranded  $\beta$ -barrel-like heterodimer with six strands in each of the two major  $\beta$ -sheets, while the models display 6:4 and 5:4  $\beta$ -strand symmetry for the human and yeast proteins, respectively. e Soluble (S), non-bound, (NB), and eluted (E) fractions of 6xHis-Sumo-Chp1 purification from E. coli coexpressing Egd1 and Egd2. Experiment was performed three times. f Total soluble cell lysate (T) of the indicated yeast strains was separated into ribosomal (R) and soluble (S) fractions and the localization of Chp1-GFP and Rpl25 among the different fractions was analyzed by western blot. Experiment was performed three times. g Polysome profile analysis of yeast cells expressing Chp1-4xFLAG after treatment with 1 mM of puromycin (green) or in the absence of treatment (black). Co-migration of Chp1-4xFLAG with ribosomes along the polysome profile fractions was analyzed by western blot. Experiment was performed two times.



Supplementary Fig. 2: Chp1 binds the GTPase domain of eEF1A. a Gene expression levels in the nonspecificbound translatome and the total translatome in reads per kilobase million (RPKM) obtained via SeRP from an untagged control yeast strain. Merged reads for the genes TEF1 and TEF2 which both encode eEF1A are shown in blue. Genes overrepresented in the Chp1-bound translatome but that also appeared overrepresented in the nonspecific matrix-bound translatome are shown in light grey. b SEC analysis of a mix of Chp1 and eEF1A domain I copurified from an E. coli system of recombinant expression. Peaks corresponding to monomeric Chp1 and Chp1- eEF1A domain I complex are marked. c Ribbon diagram of the ColabFold model of the complex between Chp1 and eEF1A domain I. Single residues of Chp1 that have been substituted by Bpa (Q18, V21, E25) for UVinducible crosslinking studies are marked and shown in pink ball and sticks (Chp1 - dark blue, eEF1A domain I cyan). d In vitro photo-crosslinking (+UV) between purified Chp1-Myc-6xHis with Bpa incorporated at amino acid positions 18, 21 or 25 and purified domain I of eEF1A. The protein mCherry was used to control for unspecific crosslinking. Proteins were analyzed by SDS-PAGE and Coomassie Brilliant Blue staining. The crosslinking product between Chp1-Myc-6xHis and eEF1A domain I is marked with an arrow. Experiment was performed three times. e STII-eEF1A domain I was coexpressed in E. coli with either 6xHis-SUMO-Chp1, 6xHis-SUMO-Chp1<sub>6A</sub> (L12A, D14A, I15A, F19A, V21A, E25), 6xHis-SUMO-Chp1<sub>10A</sub> (T3A, F4A, E7A, T8A, L12A, D14A, 115A, F19A, V21A, E25A) or 6xHis-SUMO-Chp1<sub>A2-28</sub>. Soluble fractions of the cell lysates (S) were subjected to IMAC purification and the binding of eEF1A domain I to the different mutant variants of Chp1 was analyzed by SDS-PAGE and Coomassie Brilliant Blue staining. NB (non-bound) and E (eluted) fractions from the IMAC purification. Quantifications represent means  $\pm$ SD, n=3 independent experiments using one-way ANOVA.



Supplementary Fig. 3: Chp1 binds misfolded eEF1A. a eEF1A was co-expressed with 6xHis-SUMO-Chp1 or 6xHis-SUMO in E. coli. Total cell lysate (T) was separated into pellet (P) and soluble (S) fractions and the S fraction was subjected to IMAC purification. NB (non-bound) and E (eluted) fractions from the IMAC purification. All fractions were analyzed by SDS-PAGE followed by Coomassie Brilliant Blue staining. Experiment was performed three times. b SEC analysis of a mix of Chp1 and eEF1A obtained in the E fraction of the copurification in (a). c Total cell lysate (T) from E. coli cells coexpressing STII-eEF1A and 6xHis-SUMO-Chp1 or 6xHis-SUMO was separated into soluble (S) and pellet (P) and the level of STII-eEF1A in the different fractions was analyzed by western blot. **d** Quantification of the level of recombinant STII-eEF1A in the T fraction from (c) (means  $\pm$ SD, n=3 independent experiments, two-tailed t test). e Quantification of the level of recombinant STII-eEF1A in the S fraction from (c) (means ±SD, n=3 independent experiments, two-tailed t test). f In vitro anti-RFP co-IP of Chp1mCherry and 6xHis-eEF1A (preincubated with either EDTA (10 mM), GMP-PNP (1 mM), glycerol (25% v/v), glycerol (25% v/v) and EDTA (10 mM) or glycerol (25% v/v) and GMP-PNP (1 mM). Lines between samples indicate cropping from the same gel to remove irrelevant lanes. Quantifications represent means  $\pm$ SD, n=3 (Glycerol EDTA n=3) independent experiments, one-way ANOVA. g In vitro photo-crosslinking (+UV) between Chp1-Myc-6xHis with Bpa incorporated at amino acid position 25 and 6xHis-eEF1A (preincubated with either EDTA (10 mM), GMP-PNP (1 mM) or glycerol (25% v/v)). Proteins were analyzed by SDS-PAGE and Coomassie Brilliant Blue staining. The crosslinking product between Chp1-Myc-6xHis and 6xHis-eEF1A is marked with an arrow. Quantifications represent means ±SD, n=3 independent experiments, one-way ANOVA.



Supplementary Fig. 4: *chp1* genetic interactions. a Analysis of data from TheCellMap showing significant genetic interactions that are common to *chp1* $\Delta$  and *zpr1-1* (intermediate cutoff: score < -0.08 and > 0.08 for negative and positive genetic interactions respectively, p-value < 0.05). Genes involved in translation are labeled in red. **b** Analysis of functional categories of *CHP1* negative genetic interactions. **c** Analysis of *CHP1* negative genetic interactions with genes encoding proteins identified by MS as Chp1 physical interactors involved in translation and protein quality control. Genes encoding Chp1 physical interactors that show negative genetic interaction with *CHP1* are labeled.



**Supplementary Fig. 5: eEF1A-linked phenotypes of** *chp1* $\Delta$ . **a** Growth curves of WT, *chp1* $\Delta$ , *tef1* $\Delta$ ,



Supplementary Fig. 6: Global translation in *chp1* $\Delta$  cells. a Gene expression (RPKM) in WT and *chp1* $\Delta$  cells obtained by ribosome profiling. b Average ribosome density on all coding sequences (metagene profile) of *chp1* $\Delta$  and WT cells. c *GCN4* expression (RPKM) in WT and *chp1* $\Delta$  cells obtained via ribosome profiling. Data represent means ±SD (n=2 independent experiments, two-tailed t test).



Supplementary Fig. 7: Cells lacking Chp1 display strong proteostasis defects linked to eEF1A expression. a Growth assay of WT,  $chp1\Delta$ ,  $tef1\Delta$ ,  $tef1\Delta$ ,  $chp1\Delta$ , +TEF1 and  $chp1\Delta +TEF1$  yeast strains at 30 and 39 °C on YPD plates after 3 days. b Growth assay of WT,  $chp1\Delta$ ,  $nac\Delta$  and  $chp1\Delta$   $nac\Delta$  yeast strains at 30 and 39 °C on SC plates after 3 days. c Hsf1 activity in WT,  $chp1\Delta$ ,  $nac\Delta$ ,  $chp1\Delta$   $nac\Delta$  yeast strains determined by the P<sub>CYCI</sub>-HSE-yNluc bioluminescent reporter. Data represent means  $\pm$ SD (n=4 independent experiments, one-way ANOVA). d Expression levels of Fes1 and Btn2 in WT,  $chp1\Delta$ ,  $nac\Delta$ ,  $chp1\Delta$   $nac\Delta$  yeast strains determined by western blot analysis. Quantifications represent means  $\pm$ SD (n=4 independent experiments, one-way ANOVA). e Fluorescent micrographs of Hsp104-GFP in cells with the indicated genotype. Quantification of the fraction of cells with Hsp104-GFP foci. Data represent means  $\pm$ SD (n=5 independent experiments, one-way ANOVA).



Supplementary Fig. 8: Pathogenic *EEF1A2* mutations are destabilizing and impact on eEF1A expression levels. a Human eEF1A2 pathogenic variants associated with neurodevelopmental disorders marked on a schematic representation of the human eEF1A2. Domains I, II and III are marked as well as the Switch I and II regions of the GTPase domain. b Expression levels of control V5-eEF1A (V5-TEF2) and the pathogenic variants (G70S, D91N, F98C, M102V, A125E, R264W, P331L, G382R and R421C) in WT and *chp1* $\Delta$  cells. Quantifications represent means ±SD (n=4 independent experiments). c Growth curves at 30 °C in SC medium of yeast cells expressing WT V5-eEF1A (V5-Tef2) or each of the pathogenic variants (G70S, D91N, F98C, M102V, A125E, R264W, P331L, G382R and R421C). Data represent means (n=4 independent experiments). d As in (c) but at 37 °C. e Time course of *de novo* expression of 3xHA-eEF1A from the *GAL1* promoter in WT and *chp1* $\Delta$  cells after galactose (GAL) induction. Quantifications represent means ±SD (n=4 independent experiments, two-tailed t test). f Time course of the stability of control 3xHA-eEF1A and G70S and F98C derivatives in WT cells after 3 hours of galactose induction following translational arrest with 0.1 mg/mL CHX. Quantifications represent means ±SD (n=4 independent experiments, two-tailed t test).

## Supplementary Table 1. Yeast strains.

Strain	Genotype	Source
AMY58	MATa his3A1 leu2A0 ura3A0 trp1A::kanMX chp1A::hphNT1	This work
BY4741	<i>MATa</i> his3Δ1 leu2Δ0 met15Δ0 ura3Δ0	Brachmann et al., 1998
CAY1015	MAT <b>a</b> his3A1 leu2A0 ura3A0	Gowda et al., 2013
CAY1255	MAT <b>a</b> his3D1 leu2D0 met15D0 ura3D0 HSP104-eGFP-his3MX6	EUROSCARF
CAY1363	<i>MATa</i> his3 <i>Δ1 leu2Δ0 met15Δ0 ura3Δ0 chp1Δ</i> ::kanMX	EUROSCARF
CAY1366	$MATa$ his $3\Delta 1$ leu $2\Delta 0$ ura $3\Delta 0$ chp $1\Delta$ ::kan $MX$	This work
CAY1367	MATa his3D1 leu2D0 met15D0 ura3D0 HSP104-eGFP-his3MX6 chp1A::kanMX	This work
CAY1371	MATa his3A1 leu2A0 ura3A0 CHP1-sfGFP::kanMX	This work
JQY5	MATa his3A1 leu2A0 ura3A0 CHP1-4×FLAG-kanMX	This work
JQY7	MAT <b>a</b> leu2A0 ura3A0 chp1A::kanMX his3A1::[HIS3; TEF1]	This work
JQY9	MATa leu $2\Delta 0$ ura $3\Delta 0$ his $3\Delta 1$ ::[HIS3; TEF1]	This work
JQY10	MATa his3D1 leu2D0 ura3D0 URA3-PTDH3-6×His-TEF2	This work
JQY11	MAT <b>a</b> his3D1 leu2D0 ura3D0 chp1A::kanMX URA3- <b>P</b> TDH3-6xHis-TEF2	This work
MMY09	MATa his3A1 leu2A0 ura3A0 can1A:: <b>P</b> CHP1-sfGFP-kanMX	This work
MMY105	MATa his3 $\Delta 1$ leu2 $\Delta 0$ ura3 $\Delta 0$ natNT2- $P_{GALI}$ -3xHA-TEF1	This work
MMY108	MAT <b>a</b> his3A1 leu2A0 ura3A0 chp1A::kanMX natNT2- <b>P</b> <sub>GALT</sub> -3xHA-TEF1	This work
MMY111	$MATa$ his $3\Delta 1$ leu $2\Delta 0$ ura $3\Delta 0$ tef $1\Delta$ : hphNT1	This work
MMY12	MATa his3A1 leu2A0 met15A0 ura3A0 HSP104-eGFP::hisMX6 chp1A::kanMX egd2A::natNT2	This work
MMY13	MATa his3A1 leu2A0 ura3A0 CHP1-sfGFP-kanMX egd2A::natNT2	This work
MMY130	MAT <b>a</b> his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 HSP104-yeGFP::kanMX	Verena Kohler
MMY131	MATa his3A1 leu2A0 met15A0 ura3A0 HSP104-yeGFP::kanMX chp1A::natNT2	This work
MMY132	MATa his3A1 leu2A0 met15A0 ura3A0 HSP104-yeGFP::kanMX tef1A::hphNT1	This work
MMY134	MATa his3A1 leu2A0 ura3A0 tef1A::hphNT1 chp1A::natNT2	This work
MMY139	MATa his $3\Delta 1$ leu $2\Delta 0$ met $15\Delta 0$ ura $3\Delta 0$ HSP $104$ -yeGFP::kanMX his $3\Delta 1$ :: [HIS3; TEF1]	This work
MMY14	MATa his3A1 leu2A0 met15A0 ura3A0 HSP104-eGFP-hisMX6 egd2A::natNT2	This work
MMY141	MATa his3A1 leu2A0 met15A0 ura3A0 HSP104-yeGFP::kanMX chp1A::natNT2 his3A1::[HIS3; TEF1]	This work
MMY149	MATa his3A1 leu2A0 met15A0 ura3A0 Hsp104-yeGFP::kanMX chp1A::natNT2 tef1A::hphNT1	This work
MMY17	MATa his3A1 leu2A0 met15A0 ura3A0 HSP104-eGFP-hisMX6 chp1A::kanMX egd1A::hphNT1	This work
MMY18	MATa his3A1 leu2A0 ura3A0 CHP1-sfGFP-kanMX egd1A::hphNT1	This work
MMY18	MATa his3A1 leu2A0 ura3A0 CHP1-sfGFP-kanMX egd1A::hphNT1	This work
MMY24	MATa his3A1 leu2A0 met15A0 ura3A0 HSP104-eGFP::hisMX6 chp1A::kanMX egd2A::natNT2	This work
	egd1 <i>∆</i> ::hphNT1	
MMY66	MAT <b>a</b> his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 egd2Δ::natNT2 btt1Δ::hphNT1 egd1Δ::hisMX6	This work
MMY69	MAT <b>a</b> his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 chp1Δ::kanMX egd2Δ::natNT2 btt1Δ::hphNT1 egd1Δ::hisMX6	This work
MMY89	MAT <b>a</b> his3A1 leu2A0 ura3A0 CHP1-sfGFP-kanMX egd1A::hphNT1 btt1A::hisMX6 egd2A::natNT2	This work
MMY92	MAT <b>a</b> his3Δ1 leu2Δ0 ura3Δ0 TEF1-3×HA-hphNT1	This work
MMY94	$MATa$ his $3\Delta 1$ leu $2\Delta 0$ ura $3\Delta 0$ chp $1\Delta$ ::kan $MX$ TEF1-3xHA-hphNT1	This work
MMY150	MATa his $3\Delta I$ leu $2\Delta 0$ ura $3\Delta 0$ lys $2\Delta$ ::PGAL1-HA-NatNT2-TEF2	This work
MMY152	MATa his $5\Delta I$ leu $2\Delta 0$ ura $3\Delta 0$ chp $I\Delta$ ::kanMX lys $2\Delta$ ::PGALI-HA-NatNT2-TEF2	This work
MMY 154	MATa his5A1 leu2A0 ura5A0 lys2A::PGAL1-HA-NatN12-1EF2 F98C MATa his2A1 leu2A0 ura2A0 aha1AukaaMX his2AuDCAL1 HA NatNT2 TEE2 F08C	I his work
MMV157	MATA his3A1 leu2A0 ura3A0 los2A··DGALL HA NatNT2 TEE2 G70S	This work
MMV158	MATa his341 leu240 ura340 chol A…kanMX lys24FGAL1-HA-WalNT2-TEF2 G70S	This work
1011011130	miru missari kuuzao urusao enpraminina lyszai Gabr-ira-ivutvi 2-1612 (705	THIS WOLK

## **Supplementary Table 2.** Plasmids.

Plasmid	Description	Туре	Source
ECYRS-BpA	BPa system TRP1 AmpR	2 micron	Chen et al,. 2007
pAM17	P <sub>CYCI(HSE)</sub> -yNlucPEST URA3 AmpR KanR	CEN/ARS	Masser et al,. 2016
pCA1000	P <sub>T7</sub> -lacO-6×His-SUMO-Chp1 lacI KanR	E. coli	This work
pCA1034	P T7-lacO-6×His-SUMO-Chp1-SD-StrepTag II-Egd2 lacI KanR	E. coli	This work
pCA1038	P T7-lacO-6×His-SUMO-Chp1-SD-StrepTag II-Egd2-SD-Egd1 lacI KanR	E. coli	This work
pCA1048	P T7-lacO-6×His-SUMO-StrepTag II-Egd2-SD-Egd1 lacI KanR	E. coli	This work
pJQ7	P <sub>T7</sub> -lacO-6×His-SUMO-Chp1-mCherry lacI KanR	E. coli	This work
pJQ8	P <sub>T7</sub> -lacO-6×His-SUMO-mCherry lacI KanR	E. coli	This work
pJQ12	P T7-lacO-6×His-SUMO-Chp1-SD-StrepTag II-TEF2 lacI KanR	E. coli	This work
pJQ14	P T7-lacO-6×His-SUMO-SD-StrepTag II-TEF2 lacI KanR	E. coli	This work
pJQ15	<b>P</b> <sub>T7</sub> -lacO-6×His-SUMO-Chp1-SD-StrepTag II- <i>TEF2</i> <sup>(1-238)</sup> lacI KanR	E. coli	This work
pJQ17	P <sub>T7</sub> -lacO-6×His-SUMO-SD-StrepTag II-TEF2 <sup>(1-238)</sup> lacI KanR	E. coli	This work
pJQ20	P T7-lacO-6×His-SUMO-Chp1-SD-StrepTag II-TEF2 <sup>(1-70)</sup> lacI KanR	E. coli	This work
pJQ21	P <sub>T7</sub> -lacO-6×His-SUMO-SD-StrepTag II-TEF2 <sup>(1-70)</sup> lacI KanR	E. coli	This work
pJQ22	P <sub>T7</sub> -lacO-6×His-SUMO-TEF2 <sup>(1-238)</sup> lacI KanR	E. coli	This work
pJQ24	HIS3 YIP TEF1 AmpR	YIP	This work
pJQ26	P <sub>T7</sub> -lacO-Chp1 <sup>(Q18Bpa)</sup> -Myc-6×His KanR	E. coli	This work
pJQ27	P <sub>T7</sub> -lacO-Chp1 <sup>(V21Bpa)</sup> -Myc-6×His KanR	E. coli	This work
pJQ28	P <sub>T7</sub> -lacO-Chp1 <sup>(E25Bpa)</sup> -Myc-6×His KanR	E. coli	This work
pJQ31	P T7-lacO-6×His-SUMO-StrepTag II-Egd2 <sup>(ΔUBA)-</sup> SD-Egd1 lacI KanR	E. coli	This work
pJQ32	P <sub>17</sub> -lacO-6×His-SUMO-Chp1 <sub>10A</sub> ( <sup>T3A, F4A, E7A, T8A, L12A, D14A, 115A, F19A, V21A, E25A)_ SD-StrepTag II-<i>TEF2</i><sup>(1-238)</sup> lacI KanR</sup>	E. coli	This work
pJQ33	<b>P</b> <sub>177</sub> -lacO-6xHis-SUMO- Chp1 <sub>6A</sub> <sup>(L12A, D14A, I15A, F19A, V21A, E25)</sup> -SD-StrepTag II- <i>TEF2</i> <sup>(1-238)</sup> lacI KanR	E. coli	This work
pJQ34	P <sub>17</sub> -lacO-6×His-SUMO-6xHis-SUMO-Chp1 <sup>A2-28</sup> -SD-StrepTag II- <i>TEF2</i> <sup>(1-238)</sup> lacI KanR		
p416GPD V5-TEF2	P <sub>GPD</sub> -V5-TEF2 URA3 AmpR	CEN/ARS	Carvill et al., 2020
Do. A125E	<b>P</b> <sub>GPD</sub> - <i>V5-tef2-A125E URA3</i> AmpR	CEN/ARS	Carvill et al., 2020
Do. D91N	P <sub>GPD</sub> -V5-tef2-D91N URA3 AmpR	CEN/ARS	Carvill et al., 2020
Do. F98C	P <sub>GPD</sub> -V5-tef2-F98C URA3 AmpR	CEN/ARS	Carvill et al., 2020
Do. G382R	P <sub>GPD</sub> -V5tef2-G382R URA3 AmpR	CEN/ARS	Carvill et al., 2020
Do. G70S	P <sub>GPD</sub> -V5-tef2-G70S URA3 AmpR	CEN/ARS	Carvill et al., 2020
Do. M102V	P <sub>GPD</sub> -V5-tef2-M102V URA3 AmpR	CEN/ARS	Carvill et al., 2020
Do. P331L	P <sub>GPD</sub> -V5-tef2-P331L URA3 AmpR	CEN/ARS	Carvill et al., 2020
Do. R264W	P <sub>GPD</sub> -V5-tef2-R264W URA3 AmpR	CEN/ARS	Carvill et al., 2020
Do. R421C	P <sub>GPD</sub> -V5-tef2-R421C URA3 AmpR	CEN/ARS	Carvill et al., 2020
pMM09	CHP1 <sub>L12TAG</sub> -HA-myc-8×His URA3 AmpR	CEN/ARS	This work
pMM10	CHP1 <sub>V122TAG</sub> -HA-myc-8×His URA3 AmpR	CEN/ARS	This work
pMM14	P T7-lacO-6×His-SUMO-Chp1 PT7-Egd1 lacI KanR	E. coli	This work