

Rapid high-yield expression and purification of fully post-translationally modified recombinant clusterin and mutants

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Supplementary Materials

**Supplementary Materials S1: Full length amino acid sequences of different *r*CLU constructs
(see main text Figure 2)**

Key for constructs 1-4 (twin Streptag):

MMKTLLLFVGLLLTWESGQVLG: signal peptide sequence

GGGG: 4 x glycine spacer

SAWSHPQFEKGGGSGGGSGGSAWSHPQFEK: twin Streptag sequence

1. *r*CLU- α N-ST

MMKTLLLFVGLLLTWESGQVLGDQTVS**GGGG****SAWSHPQFEKGGGSGGGSGGSAWSHPQFEKGGGG****DNELQEMSNQGSKYVNKEIQNAVNGVKQIKTLIEKTNEERKTLLSNLEEAKK**
KKEDALNETRESETKCLKELPGVCNETMMALWEECKPCLKQTCMKFYARVCRSGSGLVGR
QLEEFNLQSSPFYFWMNGDRIDSLENDRQQTHMLDVMQDHFSSRASSIIDELFQDRFFFTREP
QDTYHYLPFSLPHRRPHFFPKSRIVRSLMPFSPYEPLNFHAMFQPFLEMIHEAQQAMDIHF
HSPAFQHPPTEFIREGDDDRTVCREIRHNSTGCLRMKDQCDKCREILSVDCSTNPNPSQAKLR
RELDESLQVAERLTRKYNELLKSYQWKMLNTSSLLEQLNEQFNWVSRLANLTQGEDQYY
LRVTTVASHTSDSDVPSGVTEVVVKLFDSDPITVTPVEVSRKNPKFMETVAEKALQEYRK
KHREE

2. *r*CLU- α C-ST

MMKTLLLFVGLLLTWESGQVLGDQTVSDNELQEMSNQGSKYVNKEIQNAVNGVKQIKTL
IEKTNEERKTLLSNLEEAKKKEDALNETRESETKCLKELPGVCNETMMALWEECKPCLKQ
TCMKFYARVCRSGSGLVGRQLEEFNLQSSPFYFWMNGDRIDSLENDRQQTHMLDVMQD
HFSRASSIIDELFQDRFFFTREPQDTYHYLPFSLPHRRPHFFPK**GGGG****SAWSHPQFEKGGG**
SGGGSGGSAWSHPQFEKGGG**RIVRSLMPFSPYEPLNFHAMFQPFLEMIHEAQQAMDIHF**
HSPAFQHPPTEFIREGDDDRTVCREIRHNSTGCLRMKDQCDKCREILSVDCSTNPNPSQAKLR
RELDESLQVAERLTRKYNELLKSYQWKMLNTSSLLEQLNEQFNWVSRLANLTQGEDQYY
LRVTTVASHTSDSDVPSGVTEVVVKLFDSDPITVTPVEVSRKNPKFMETVAEKALQEYRK
KHREE

3. *r*CLU-βN-ST

MMKTLLLFVGLLLTWESGQVLGDQTVSDNELQEMSNQGSKYVNKEIQNAVNGVKQIKTL
IEKTNEERKTLLSNLEEAKKKKEDALNETRESETKCLKELPGVCNETMMALWEECKPCLKQ
TCMKFYARVCRSGSGLVGRQLEEFLNQSSPFYFWMNGDRIDSLENDRQQTHMLDVMQD
HFSRASSIIDELFQDRFFTREPQDTYHYLPFSLPHRRPHFFFPKSRIVRSGGGGSAWSHPOFE
KGGGSGGGSGGSAWSHPOFEKGGGGLMPFSPYEPLNFHAMFQPFLEMIHEAQQAMDIH
FHSPAFQHPPTEFIREGDDDRTVCREIRHNSTGCLRMKDQCDKCREILSVDCSTNNPSQAKL
RRELDLQVAERLTRKYNELLKSYQWKMLNTSSLLEQLNEQFNWWSRLANLTQGEDQY
YLRVTTVASHTSDSDVPSGVTEVVVKLFDSDPITVTPVEVSRKNPKFMETVAEKALQEYR
KKHREE

4. *r*CLU-βC-ST

MMKTLLLFVGLLLTWESGQVLGDQTVSDNELQEMSNQGSKYVNKEIQNAVNGVKQIKTL
IEKTNEERKTLLSNLEEAKKKKEDALNETRESETKCLKELPGVCNETMMALWEECKPCLKQ
TCMKFYARVCRSGSGLVGRQLEEFLNQSSPFYFWMNGDRIDSLENDRQQTHMLDVMQD
HFSRASSIIDELFQDRFFTREPQDTYHYLPFSLPHRRPHFFFPKSRIVRSLMPFSPYEPLNFHA
MFQPFLEMIHEAQQAMDIHFHSPAFQHPPTEFIREGDDDRTVCREIRHNSTGCLRMKDQCD
KCREILSVDCSTNNPSQAKLRRELDLQVAERLTRKYNELLKSYQWKMLNTSSLLEQLNE
QFNWWSRLANLTQGEDQYYLRVTTVASHTSDSDVPSGVTEVVVKLFDSDPITVTPVEVS
RKNPKFMETVAEKALQEYRKKHREEGGGGSAWSHPOFEKGGGSGGGSGGSAWSHPOF
EK

Key for construct 5 (C-tag):

MMKTLLLFVGLLLTWESGQVLG: signal peptide sequence

EPEA: C-tag

5. rCLU-CT:

MMKTLLLFVGLLLTWESGQVLGDQTVSDNELQEMSNQGSKYVNKEIQNAVNGVKQIKTL
IEKTNEERKTLLSNLEEAKKKKEDALNETRESETKLLKELPGVCNETMMALWEECKPCLKQ
TCMKFYARVCRSGSLVGRQLEEFLNQSSPFYFWMNGDRIDSLENDRQQTHMLDVMQD
HFSRASSIIDELFQDRFFTREPQDTYHYLPFSLPHRRPHFFFPKSRIVRSLMPFSPYEPLNFHA
MFQPFLEMIHEAQQAMDIHFHSPAFQHPPTEFIREGDDDRTVCREIRHNSTGCLRMKDQCD
KCREILSVDCSTNNPSQAKLRRELDLQVAERLTRKYNELLKSYQWKMLNTSSLLEQLNE
QFNWVSRLANLTQGEDQYYLRVTTVASHTSDSDVPSGVTEVVVKLFDSDPITVTPVEVS
RKNPKFMETVAEKALQEYRKKHREEEPEA

Supplementary Materials S2:

Immuno dot blot detection of *rCLU-CT* secreted by MEXi 293E cells

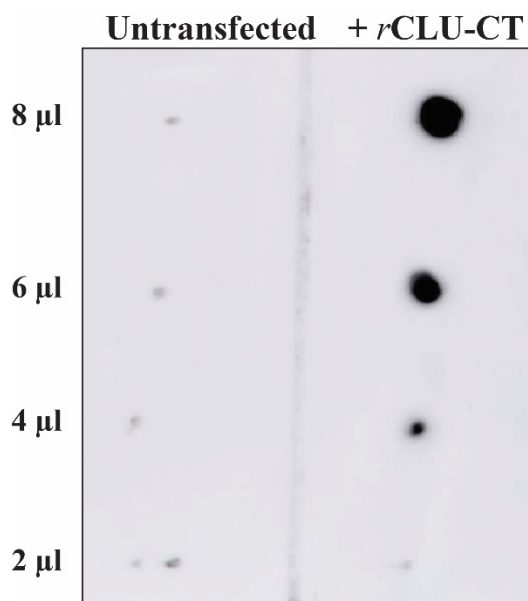


Figure S2 Immuno dot blot detection of *rCLU-CT* secreted by MEXi 293E cells. Culture supernatant (CS) from MEXi 293E cells overexpressing *rCLU-CT* was harvested on day 5 post transfection. Different volumes of CS (indicated at left side of image) taken from untransfected cells (grown for 6 days, left) or transfected cells expressing *rCLU-CT* (right) were spotted onto a nitrocellulose membrane. Specific immunodetection of *rCLU-CT* was performed as described in Methods. The image shown is representative of 2 independent experiments.

Supplementary Materials S3:

Complete SDS PAGE images corresponding to manuscript Figures 3B, 4A and 7A

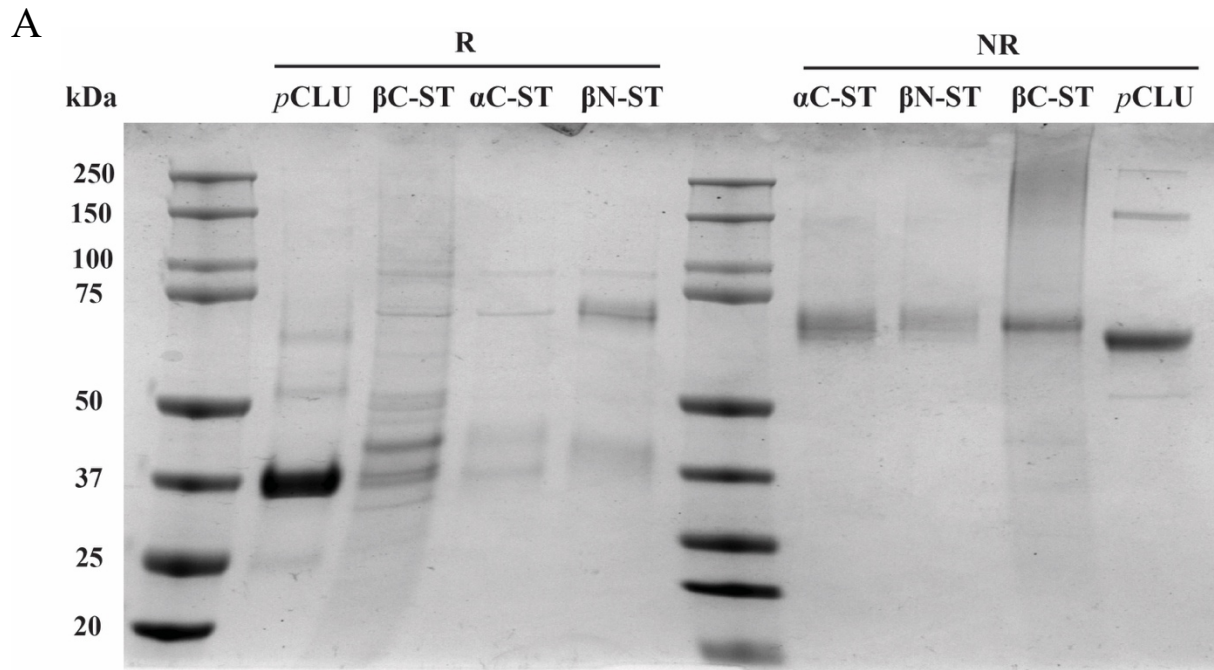


Figure S3A. Complete image of SDS PAGE of purified *r*CLU- β N-ST, *r*CLU- α C-ST, *r*CLU- β C-ST and *p*CLU. The molecular mass of protein standards (left lane) are shown in kDa. Reduced (R) and Non-reduced (NR) lanes are indicated above the image. Both panels shown in main text Figure 3B have been taken from this image.

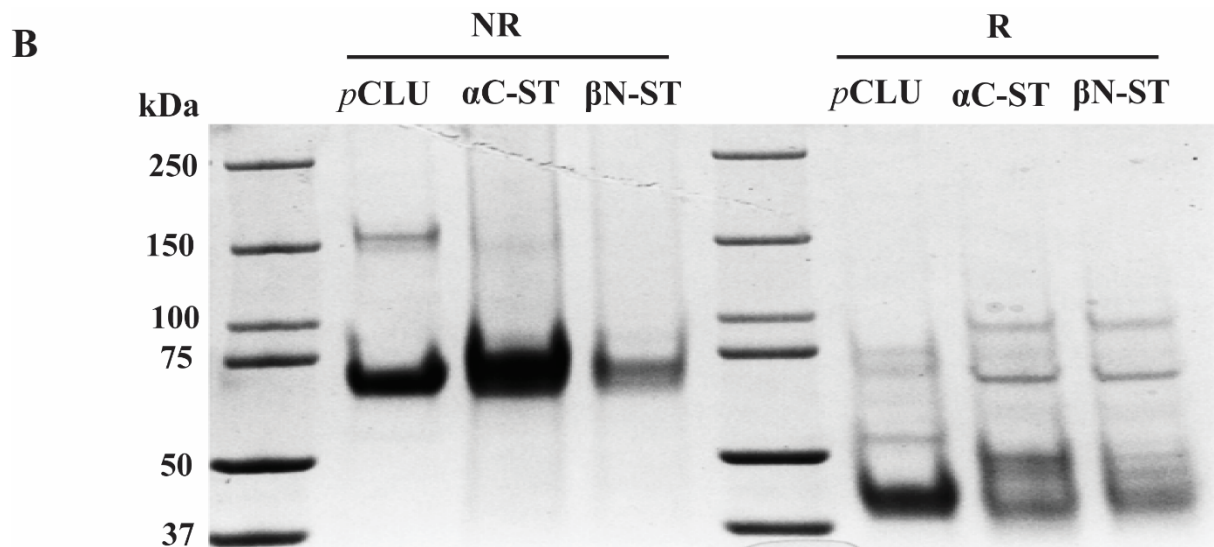


Figure S3B. Complete image of SDS PAGE analysis of purified *p*CLU, *r*CLU- α C-ST and *r*CLU- β N-ST under non-reducing (NR) and reducing (R) conditions. The molecular mass of protein standards (left lane) are shown in kDa. The left and middle panels in main text Figure 4A are taken from this image.

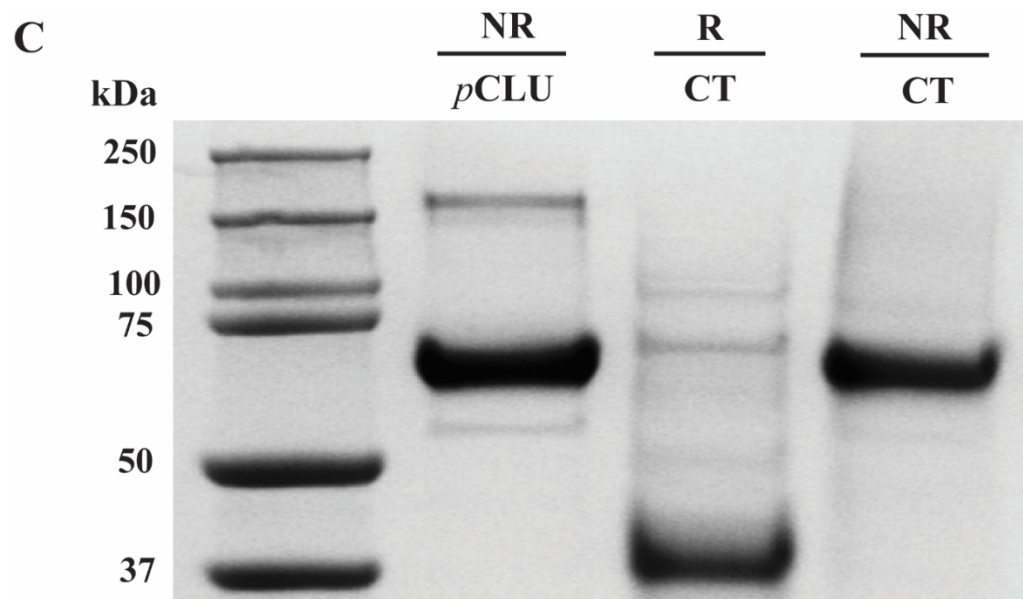


Figure S3C. Complete image of SDS PAGE analysis of purified *p*CLU, *r*CLU-CT under non-reducing (NR) and reducing conditions (R). The molecular mass of protein standards (left lane) are shown in kDa. The right panel in main text Figure 4A is taken from this image.

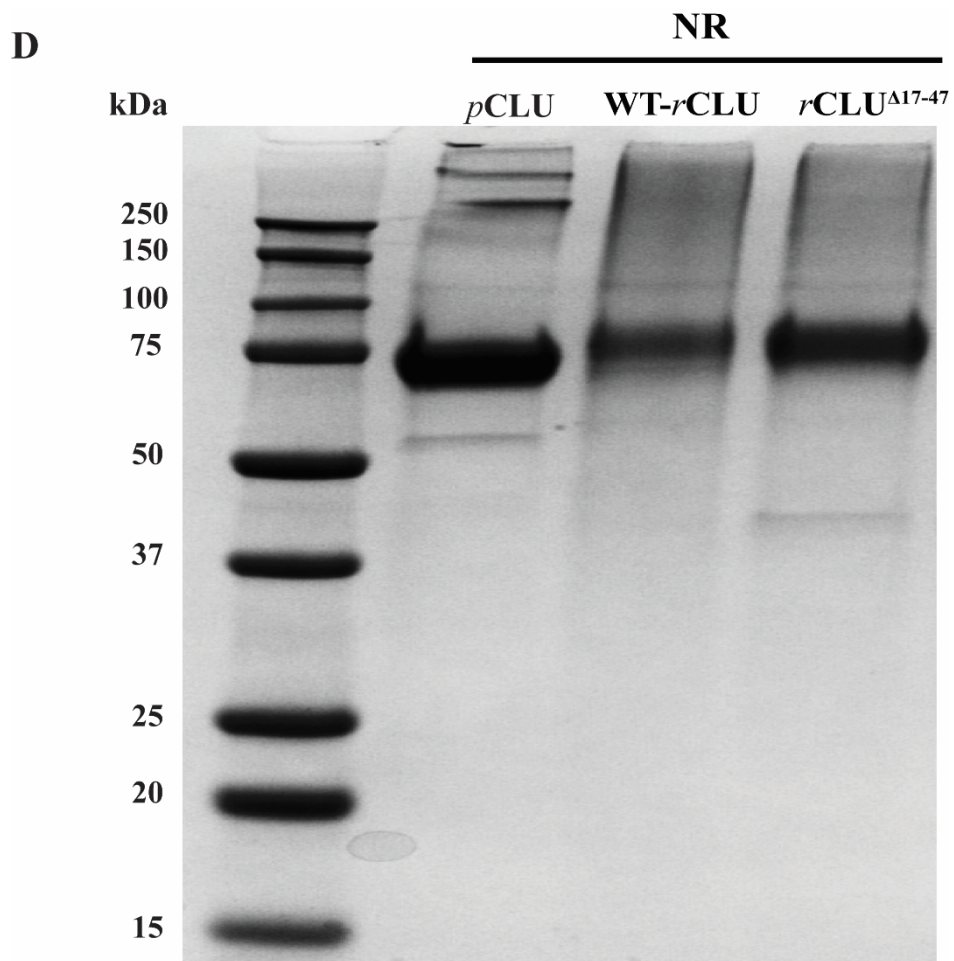


Figure S3D. Complete image of SDS-PAGE analyses of wild type and mutant *r*CLU under non-reducing conditions (NR). *p*CLU, full length WT-*r*CLU- α C-ST (WT-*r*CLU) and a CLU deletion mutant *r*CLU^{Δ17-47}- α C-ST (in which residues 17-47 were deleted) were analysed by SDS PAGE. The molecular mass of protein standards (left lane) are shown in kDa. The left panel in main text Figure 7A is taken from this image.

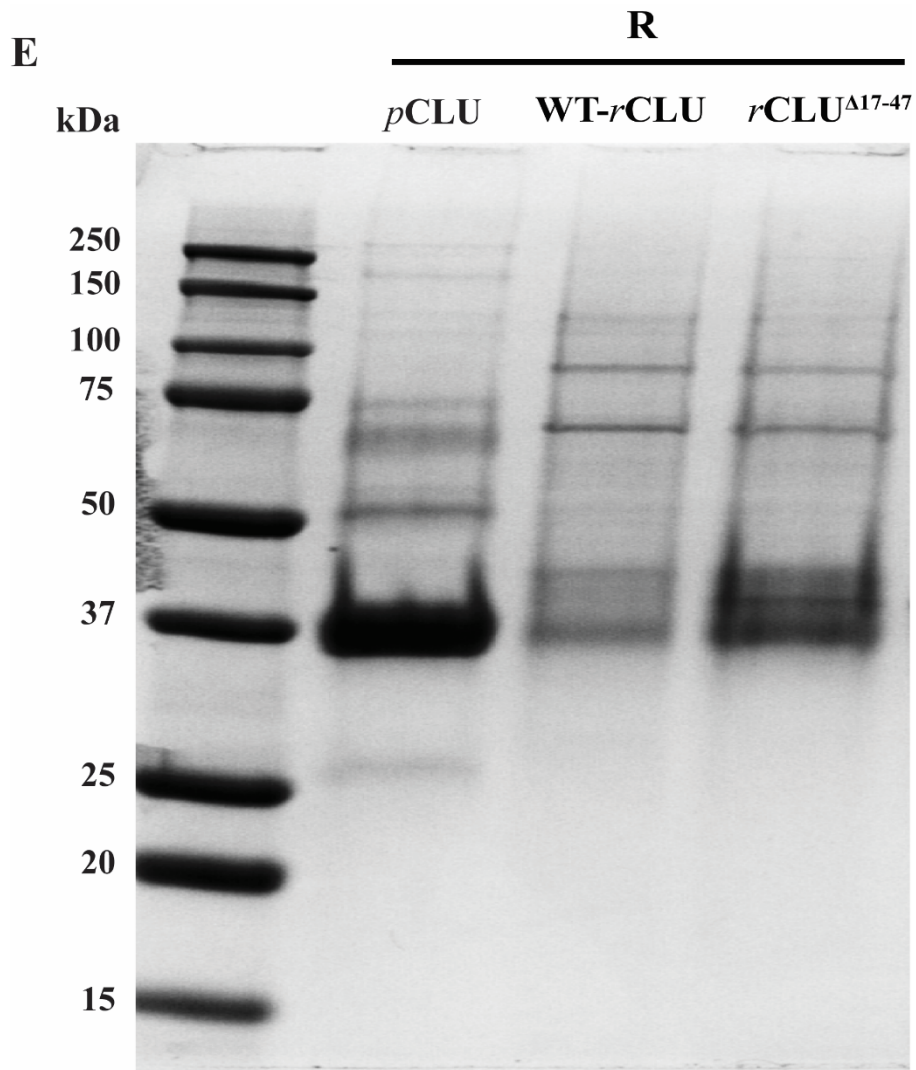


Figure S3E. Complete image of SDS-PAGE analyses of wild type and mutant *r*CLU under reducing conditions (R). *p*CLU, full length WT-*r*CLU- α C-ST (WT-*r*CLU) and a CLU deletion mutant *r*CLU^{Δ17-47}- α C-ST (in which residues 17-47 were deleted) were analysed by SDS PAGE. The molecular mass of protein standards (left lane) are shown in kDa. The right panel in main text Figure 7A is taken from this image.