

Appendix for

Reprogramming of the ubiquitin ligase Ubr1 by intrinsically disordered Roq1 through cooperating multifunctional motifs

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Appendix Table S1 - Oligonucleotides used in this study.

Oligonucleotide	Sequence
Pho8* fw	ttaagaaggagatatacatatgtctgcatcacacaagaagaag
Pho8* rev	ctcagtggtggtggtggtggtgtatttctgtagcatcaaaatctg
His ₆ -SUMO-Roq1(22-104)(R22A) fw	tggtcccgtggtctgcaccaccaatctgttc
His ₆ -SUMO-Roq1(22-104)(R22A) rev	gaacagattggtggtgagccagcgggacca
KGEQ replacement fw	tcacagagaacagattggtgtaggagcaaggggaagaactgttcacgggtg
KGEQ replacement revPhe-L-GFP rev	cacccgtgaacagttcttccccctgtctcctaccaccaatctgttctctgtga
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Phe-L-GFP fw	ggctcacagagaacagattggtggttcagcaagggggaaga
Cup9 fw	gttctgactatgcgaattataactgcgaatacaaaacagg
Cup9 rev	gtggtggtggtggtgattcatatcagggttgatagc
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Pho8* MBP fw	atcagattttagtctacagaaataatgaaatcgaagaaggtaaactgg
Pho8* MBP rev	agctcgaattcgatccggtcttcaagtctgcgcgtcttcag
Pho8* MBP open fw	tgaagaccgatccgaattc
Pho8* MBP open rev	tatttctgtagcatcaaaatctgatg
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Pho8* intern seq fw	aagtcaaggtggcttggg
MBP intern seq. rev	ttgtaacgtacggcatccc
MBP intern seq. fw	aaatcatgccgaacatccc
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Pho8 rev	ctccaaaacgtattgaaatgcttcatcgaaagccaac
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Roq1(22-60)-HA fw	taccatacagatgttctgactatgctggaactaatttaggcatacaggaaaacacaagc
Roq1(22-60)-HA rev	cagctctacaaagtagtatatcacgcc
pho-HA-tag fw	taccatacagatgttctgactatgctgaggatccgaattcgagct
FLAG-Ubr1 His removal fw	taactcgagatcatgtaattagttatgtcacgc
FLAG-Ubr1 His removal rev	ccaaatctctcgtcatcagagtc
Ubr1 (I687D) rev	ggaggtggtatattctaaataggagtcgaaattttagtctcgtgaagaaca
Ubr1 (I687D) fw	tgttctcacgaagatcaaaatttcgactcctatttagaatataccacctcc
Ubr1 (Y823D) rev	taattcgggattgtttataatccgatgcttgatgaacacagaca
Ubr1 (Y823D) fw	tgtctgtttacatcaagcatcggattataaaaacaatcccgaatta
MBP intern seq. rev	ttgtaacgtacggcatccc
HA-term fw	taccatacagatgttctgactatgctgaggatccgaattcgagct
Roq1-HA rev	cgcatagtcaggaacatcgtatgggtatgaacaacggcgagagtga
His-SUMO-Roq1 fw	tgaggatccgaattcgagct
His-SUMO-Roq1 rev	tgaacaacggcgagagtga
Open FLAG-Ubr1 Chk1 rev	gtcgactagaggatccccg

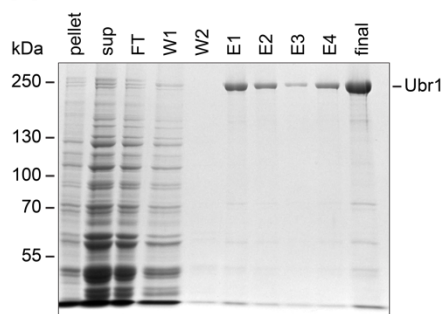
Open FLAG-Ubr1 Chk1 fw	taactcgagatcatgtaattagttatgtcacgc
Chk1-ALFA-FLAG fw	atgagtctctcgcagggtgcacctttacccc
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Roq1_I54K fw	gctctacaaagtagtatttcacgccgccag
Roq1_I54K rev	acacagcctggcgtgaaatactac
FLAG fw_new	gattacaaggacgacgatgacaag
ADH rev_new	tctagagcggccagcttgaggt
Ubr1 fw	tccgttgctgatgatgattaggt
Roq1-22 fw	aggagccagcgggacca
Ubi-Roq1-22 rev	ctgggtcccgtggctcctaccacctcttagccttagca
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Roq1_V58E fw	gcctaaattagtcagctcttcaaagtagtatatcacgccca
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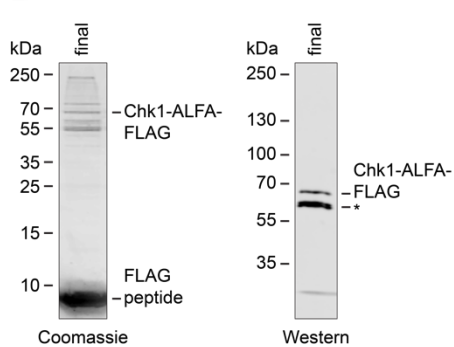
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Appendix Figure S1

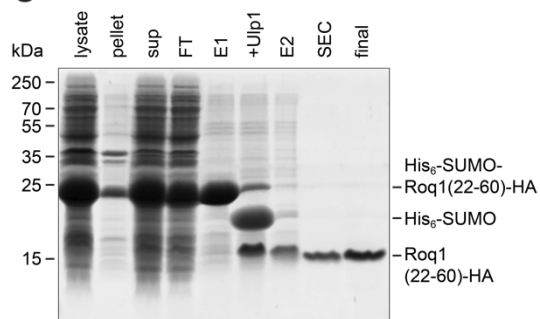
A Ubr1 purification



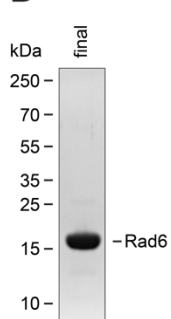
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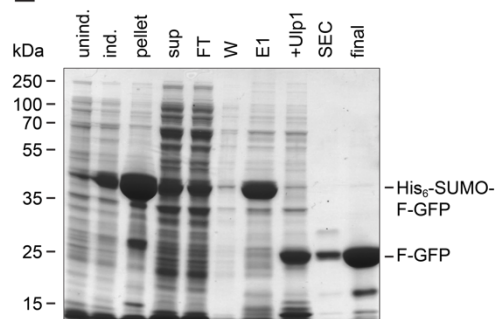
C Roq1 purification



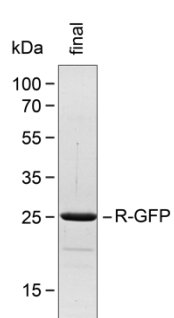
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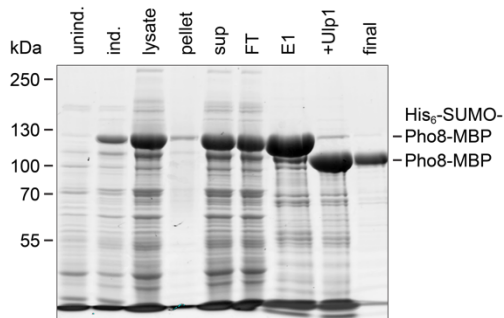
E purification F-GFP



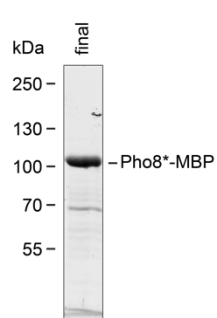
F purified R-GFP



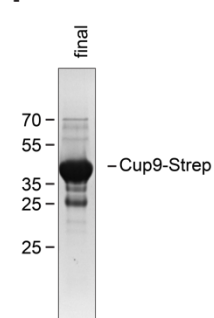
G Pho8 purification



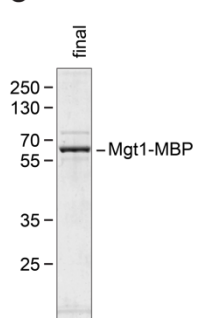
H purified Pho8*



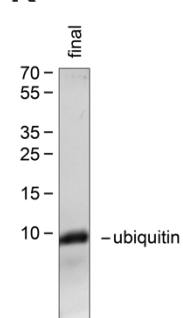
I purified Cup9



J purified Mgt1



K purified ubiquitin



Appendix Figure S1. Isolation and purity of recombinant proteins.

- A Coomassie-stained SDS-PAGE gel of FLAG-Ubr1 purification. Shown are the pellet (pellet) and supernatant (sup) of the centrifuged yeast lysate, the flow-through after incubation of the supernatant with anti-FLAG beads (FT), the washes of the anti-FLAG beads (W1, W2), the protein-containing fractions resulting from the elution with FLAG peptide (E1-E4) and the purified FLAG-Ubr1 (final).
- B Coomassie-stained SDS-PAGE gel (left) and western blot (right) of purified Chk1-ALFA-FLAG. The purification of Chk1 followed the same strategy as that of Ubr1. The band of FLAG peptide in the Coomassie-stained gel stems from the elution from the anti-FLAG beads. The asterisk (*) denotes a degradation product that arose during expression or purification.
- C Coomassie-stained SDS-PAGE gel of Roq1 purification, with Roq1(22-60)-HA as an example. Shown are the lysate of bacteria expressing His₆-SUMO-Roq1(22-60)-HA (lysate), the pellet (pellet) and supernatant (sup) of the centrifuged lysate, the flow-through (FT) and eluate (E1) of the immobilized metal affinity chromatography with nickel beads, the eluate after incubation with Ulp1 protease to cleave off His₆-SUMO (+Ulp1), the eluate after the second immobilized metal affinity chromatography with nickel beads (E2), the eluate of the size exclusion chromatography (SEC) and the purified Roq1(22-60)-HA (final).
- D Coomassie-stained SDS-PAGE gel of purified Rad6. The purification of Rad6 followed the same strategy as that of Roq1.
- E Coomassie-stained SDS-PAGE gel of F-GFP purification. Shown are the lysate of bacteria before and after induction of His₆-SUMO-F-GFP expression (unind. and ind.), the pellet (pellet) and supernatant (sup) of the centrifuged lysate, the flow-through (FT), wash (W) and eluate (E1) of the immobilized metal affinity chromatography with nickel beads, the eluate after incubation with Ulp1 protease to cleave off His₆-SUMO (+Ulp1), the eluate of the size exclusion chromatography (SEC) and the purified F-GFP (final).
- F Coomassie-stained SDS-PAGE gel of purified R-GFP. The purification of R-GFP followed the same strategy as that of F-GFP.
- G Coomassie-stained SDS-PAGE gel of Pho8-MBP purification. Shown are the lysed bacteria before induction (unind.) and after induction of His₆-SUMO-Pho8-MBP expression (ind.), the cell lysate used for purification (lysate), the pellet (pellet) and supernatant (sup) of the centrifuged lysate, the flow-through (FT) and eluate from the immobilized metal affinity chromatography with nickel beads (E1), the eluate after incubation with Ulp1 protease to cleave off His₆-SUMO (+Ulp1) and the eluate of the size exclusion chromatography (final).
- H Coomassie-stained SDS-PAGE gel of purified Pho8*-MBP. The purification Pho8* followed the same strategy as that of Pho8.
- I Coomassie-stained SDS-PAGE gel of purified Cup9-Strep. The purification of Cup9 followed the same strategy as that of Roq1.
- J Coomassie-stained SDS-PAGE gel of purified Mgt1-MBP. The purification of Mgt1 followed the same strategy as that of Roq1.
- K Coomassie-stained SDS-PAGE gel of purified ubiquitin.