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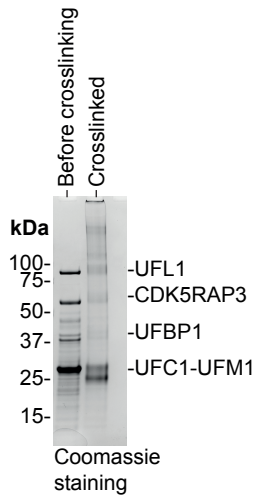
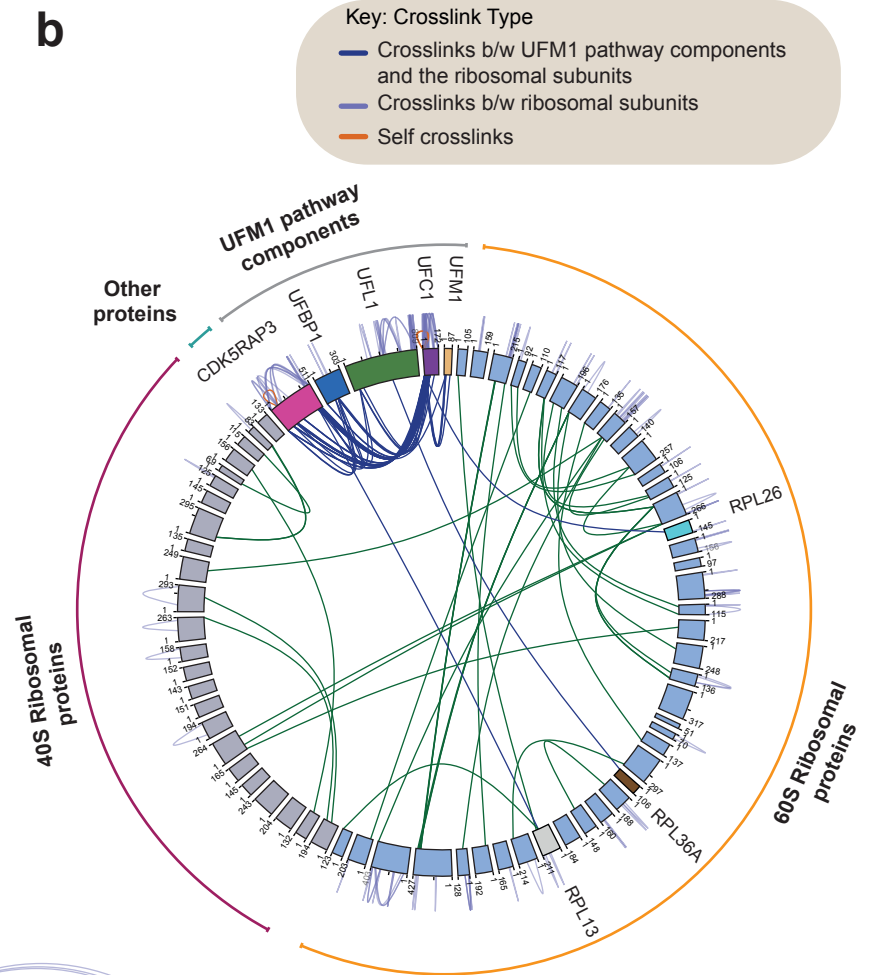
**Supplementary information**

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**The UFM1 E3 ligase recognizes and releases  
60S ribosomes from ER translocons**

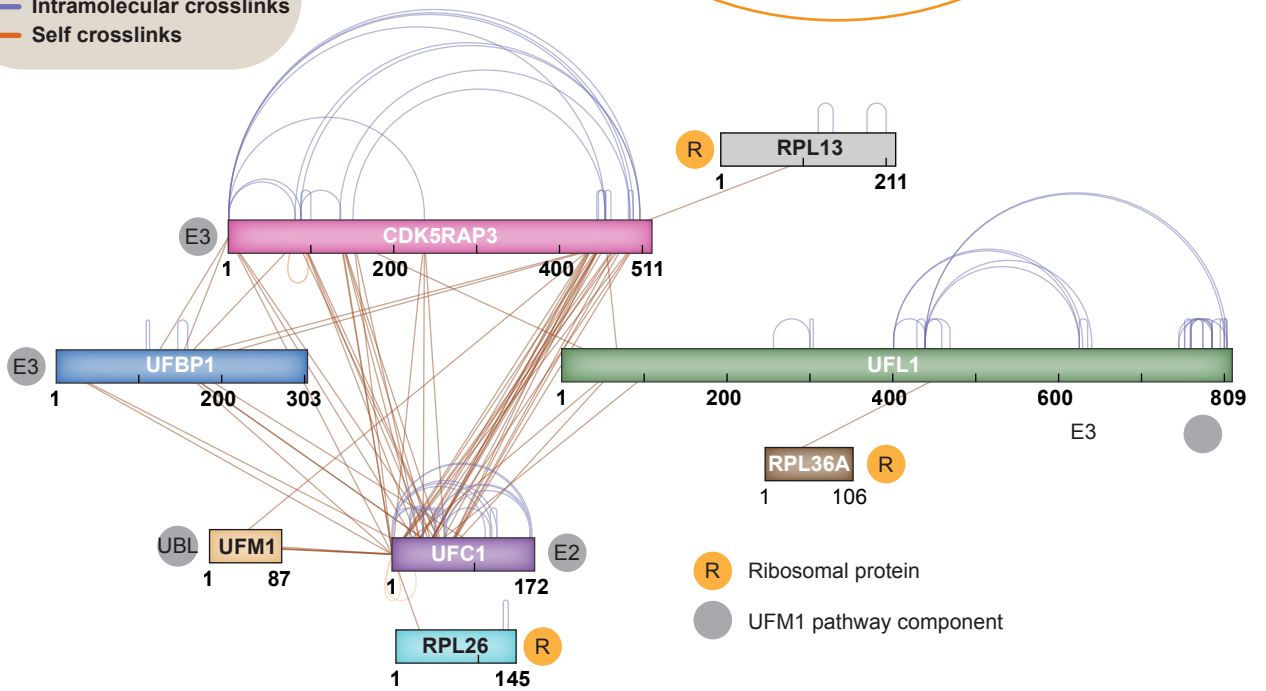
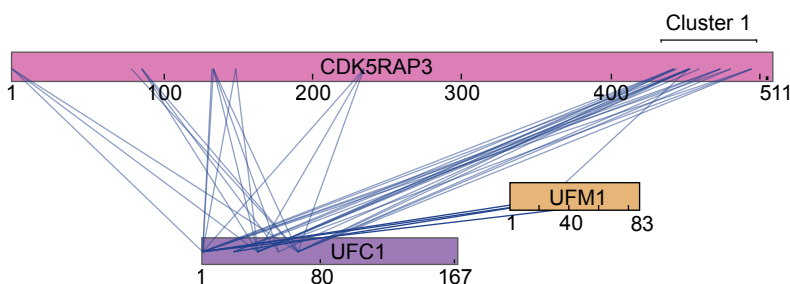
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In the format provided by the  
authors and unedited

**a****b****c**

Key: Crosslink Type

- Intermolecular crosslinks
- Intramolecular crosslinks
- Self crosslinks

**d**

### **Supplementary Figure 1 Summary of crosslinking-MS data**

**a**, Coomassie-stained SDS-PAGE gel analysing formation of UREL-ribosome complexes in the presence and absence of the crosslinker disuccinimidyl dibutyric urea (DSBU). The protein complexes were incubated with DSBU for 30 min at 23 °C. The crosslinking reaction was quenched by adding excess Tris (pH 8) and run on a 4-12% SDS-PAGE gel followed by Coomassie staining. **b**, Circular plot showing crosslinks formed between UREL, UFC1-UFM1 and ribosomal subunits. The ligase components specifically interact with the 60S ribosomal subunits. **c**, Crosslinks between UREL, UFC1-UFM1 and the ribosomal subunits derived from above are shown to highlight the interaction network. **d**, Schematic showing intermolecular crosslinks between UFC1, UFM1 and CDK5RAP3 identified by crosslinking mass spectrometry (XL-MS).

**Supplementary Table 1. Details of antibodies and recombinant proteins**

<b>Antibodies and dilution used</b>		
Anti-UFM1 (1:1000)	Abcam	ab109305
Anti-UBA5 (1:1000)	Universal Biologicals	A304-115A-T
Anti-UFC1 (1:1000)	Abcam	ab189252
Anti-UFL1 (1:1000)	Abcam	ab227506
Anti-CDK5RAP3 (1:1000)	Bethyl Laboratories	A300-870A
Anti-rabbit IgG, HRP-linked Antibody (1:10,000)	CST	70745
IRDye 800CW anti-Rabbit (1:10,000)	Li-COR	926-32211
IRDye 680CW anti-Rabbit (1:10,000)	Li-COR	926-68071
Anti-UFBP1 (DDRKG1) (1:1000)	Proteintech	21445-1-AP
Anti-UFSP2 (1:1000)	Abcam	ab192597
Anti-RPL26 (1:1000)	Abcam	ab59567
Anti-ERp72 (1:1500)	CST	5033T
Anti-GAPDH (1:5000)	Abcam	ab8245
Anti-RPS10 (1:1000)	Abcam	ab151550
Anti-RPL10A (1:5000)	Abcam	ab174318
Anti-ODR4 (1:1000)	Abcam	Ab121495
<b>Recombinant proteins</b>		
6xHis-TEV-UFL1/StrepII-UFBP1 29-C K193A	This Study	DU76181
6xHis-TEV-UFL1 /StrepII-UFBP1 29-C F196A	This Study	DU76182
6xHis-TEV-UFL1 /StrepII-UFBP1 29-C K193D	This Study	DU76187
6xHis-TEV-UFL1 StrepII-UFBP1 29-C V198R	This Study	DU76188
6xHis-TEV-UFL1 /StrepII-UFBP1 29-C F196R	This Study	DU76190
6xHis-TEV-UFL1 /StrepII-UFBP1 29-C	Peter et al. <sup>3</sup>	DU63479
6xHis-3C-UFM1 1-83	Peter et al. <sup>3</sup>	DU59441
6xHis-3C-UFM1 1-83 K69R	Peter et al. <sup>3</sup>	DU59446
6xHis-3C-UFM1 1-83 K0	Peter et al. <sup>3</sup>	DU59472
GST-3C-UFBP1 178-204	This Study	DU75665
GST-3C-UFC1	This Study	DU73281
GST-3C-UFC1 L32R	This Study	DU75492
GST-3C-UFC1 I40R	This Study	DU75493
GST-3C-UFC1 D50A	This Study	DU75504

GST-3C-UFC1 C116K	This Study	DU73986
6xHis-TEV-UFM1 1-83	This Study	DU73256
6xHis-UFL1 1-179/UFBP1 207-C	This Study	DU72757
6xHis-UFL1 1-179 I8R/UFBP1 207-C	This Study	DU75507
6xHis-UFL1 1-179 L11R/UFBP1 207-C	This Study	DU75508
6xHis-UFL1 1-179 F15R/UFBP1 207-C	This Study	DU75509
6xHis-UFL1 1-179 Q19R/UFBP1 207-C	This Study	DU75505
6xHis-UFL1 1-179/UFBP1 207-C R265A	This Study	DU75506
6xHis-UFL1 22-179/Strep-UFBP1 207-C	This Study	DU75115
6xHis-TEV-UFL1 1-179/UFBP1 174-C	This Study	DU75543
6xHis-TEV-UFL1 1-179 del21-24/UFBP1 174-C	This Study	DU75672
6xHis-TEV-UFL1 1-179 del25-28/UFBP1 174-C	This Study	DU75673
6xHis-TEV-UFL1 1-179/UFC1 C116K-UFBP1 204-C	This Study	DU75485
6xHis-3C-UBA5	Peter et. al. <sup>3</sup>	DU32106
8xHis-MBP-3C-CDK5RAP3	Peter et. al. <sup>3</sup>	DU59674