

Supplementary information for:

**Interdomain assembly between the fungal tRNA ligase
adenylyltransferase and kinase domain**

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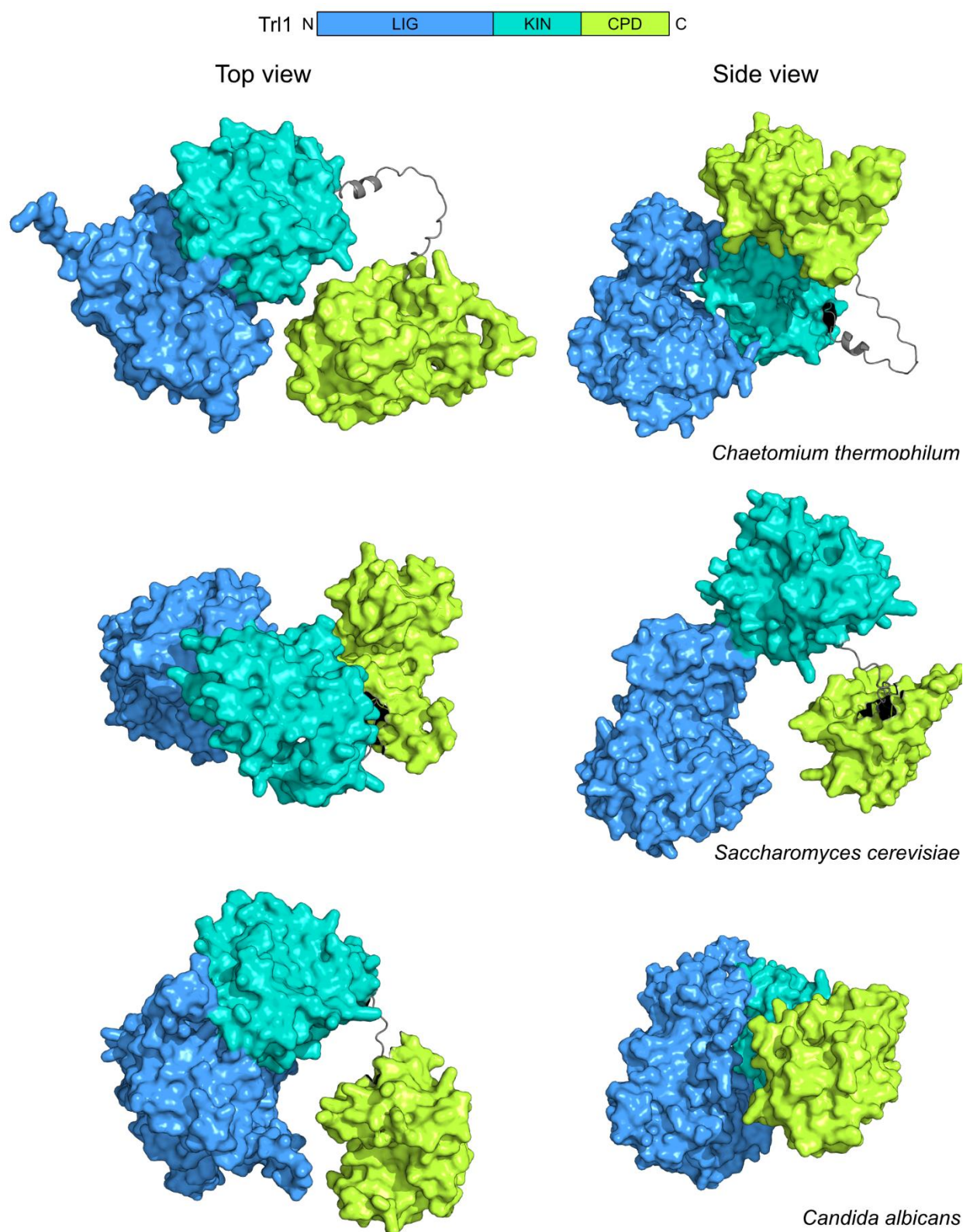


FIGURE S1: AF3 predictions of Trl1 from different fungal species. Top and side view of the AF3 predicted tripartite Trl1 structures from *Chaetomium thermophilum*, *Saccharomyces cerevisiae* and *Candida albicans* are shown as surface representation. The LIG, KIN and CPD domains are colored as indicated in the top scheme.

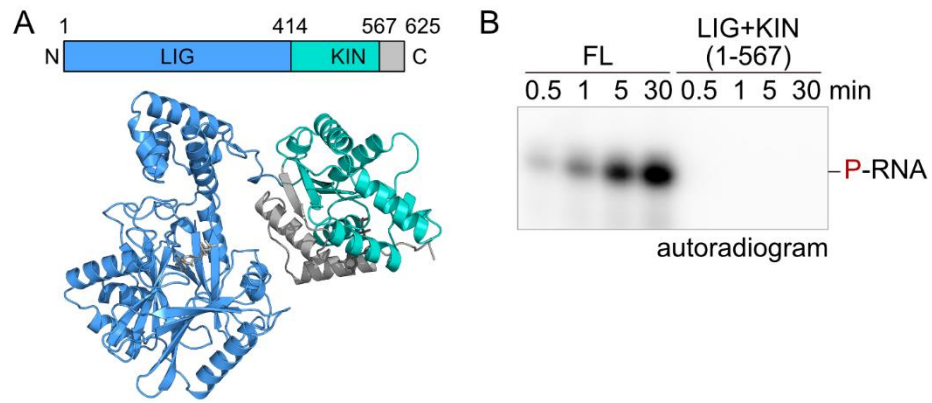


FIGURE S2: CtTrl1-LIG-KIN (1-567) construct and activity assay.

(A) Overview of CtTrl1-LIG-KIN-trunc (1-567) truncation construct. The LIG is shown in blue, the polynucleotide KIN in cyan and the truncated part in grey. The residue numbering refers to the domain boundaries.

(B) RNA phosphorylation by FL or LIG-KIN-trunc (1-567) variant (1 μ M). The autoradiogram shows formation of P^{32} -phosphorylated ASL-4U RNA (P-RNA) over time.

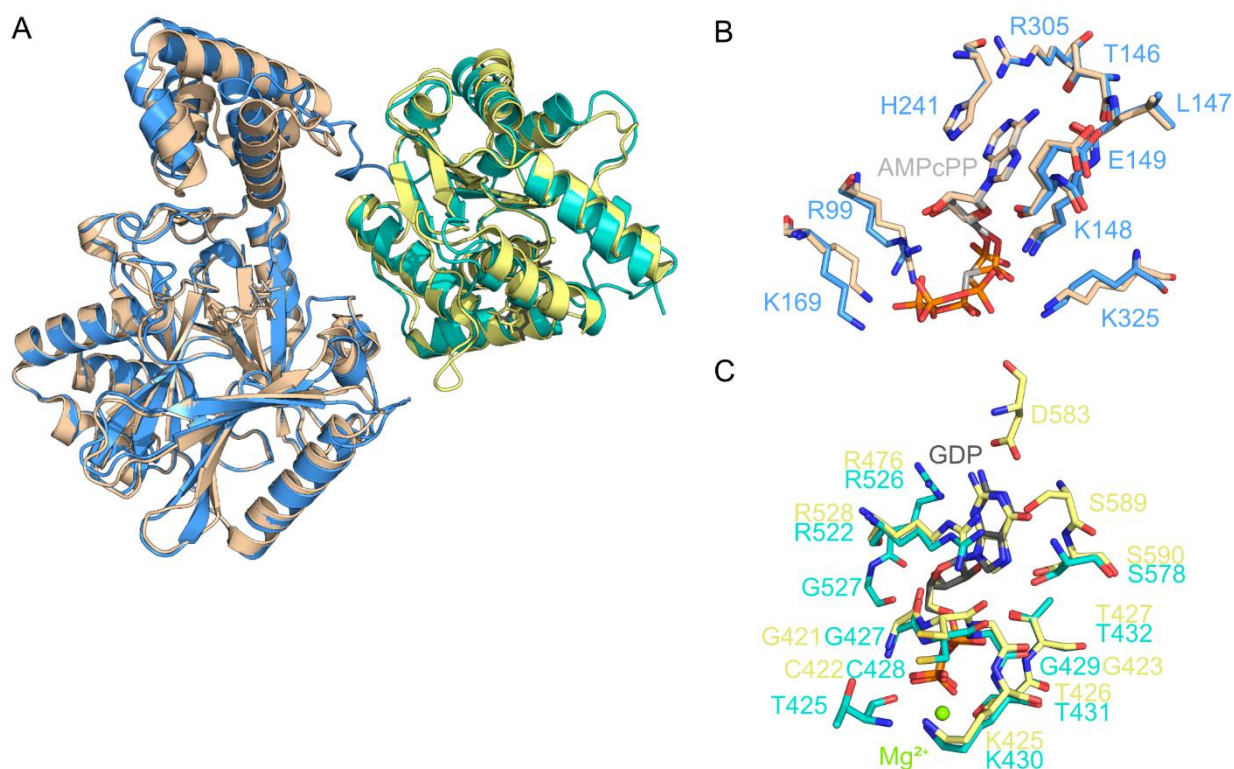


FIGURE S3: Superposition of CtTrl1-LIG-KIN complex structure with CtTrl1-LIG and CaTrl1-KIN structures single domain structures.

(A) Overview cartoon representation of the CtTrl1-LIG-KIN structure superimposed to the CtTrl1-LIG•AMPcPP structure (orange, PDB ID 6N67, RMSD=0.540) and the CaTrl1-KIN•GDP structure (yellow, PDB ID 5U32, RMSD=1.804).

(B) Zoom into the AMPcPP coordination of the superimposed CtTrl1-LIG-KIN (blue amino acid residues, grey AMPcPP) and CtTrl1-LIG•AMPcPP (orange) structures shown as sticks and colored by element.

(C) Zoom into the GDP coordination of the superimposed CtTrl1-LIG-KIN (cyan amino acid residues, grey GDP) and CaTrl1-KIN•GDP (yellow) structures shown as sticks and colored by element.

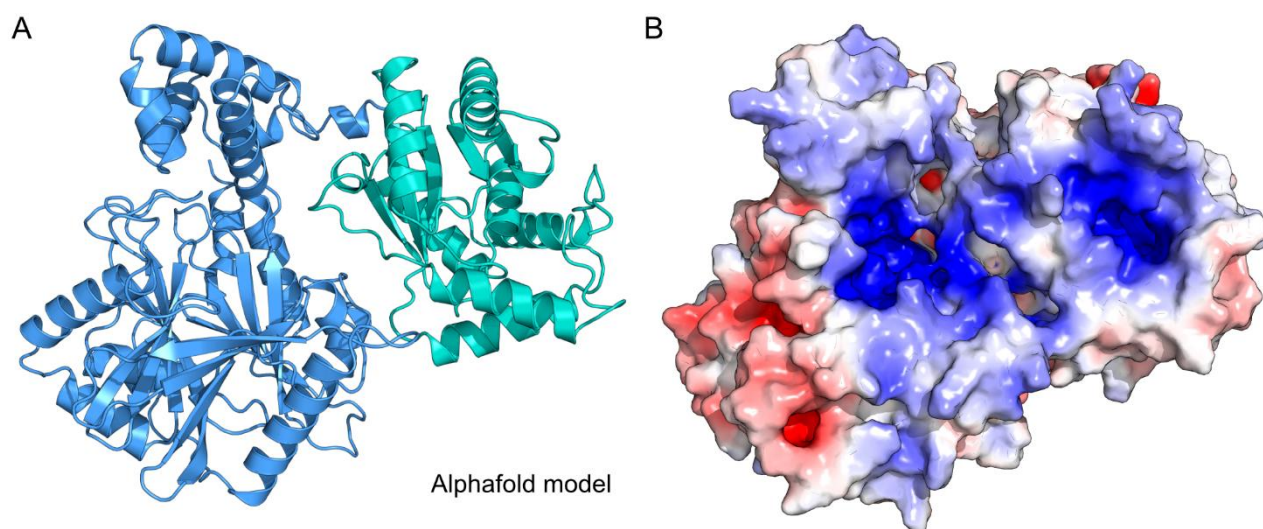


FIGURE S5: AF3 prediction of the *CtTrl1*-LIG-KIN construct.

(A) Cartoon representation of the AF3 predicted *CtTrl1*-LIG-KIN model. The LIG domain is colored in blue and the KIN domain in cyan.

(B) Electrostatic surface potential map of *CtTrl1*-LIG-KIN from A. Positive potential is represented in blue and negative potential in red.

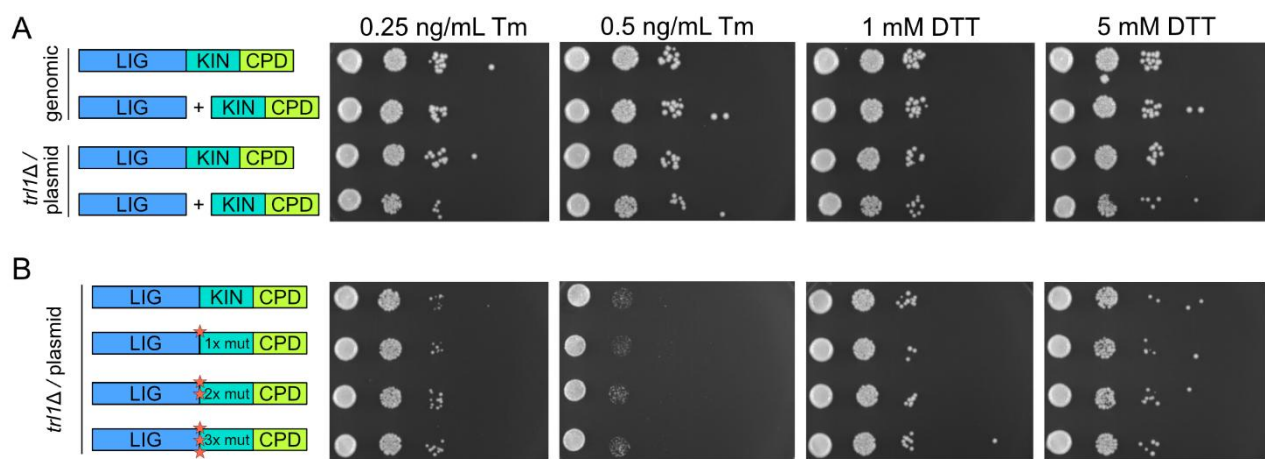


FIGURE S6: Impact of LIG-KIN interaction on yeast survival under ER stress conditions.

(A) Growth assay of the indicated yeast strains expressing ScTrl1-LIG separated from ScTrl1-KIN+CPD domains. Dilution series (10-fold) of yeast cells were spotted on the indicated selective solid media and incubated for 2 days at 30°C. This assay was performed as duplicate (n=2); the raw data is available at <https://doi.org/10.5281/zenodo.17020134>.

(B) Growth assay of plasmid-based LIG/KIN interface mutants (single mutation: 1x mut, D545K; double mutation: 2x mut, D545K, K579E; triple mutation: 3x mut, D545K, K579E, E587K) in the $\Delta trl1$ strain compared to the plasmid-based expressed ScTrl1 WT. The red stars indicate the amount of mutations at the interface site. Dilution series (10-fold) of yeast cells were spotted on the indicated selective solid media and incubated for 2 days at 30°C. This assay was performed as triplicate (n=3); the raw data is available at <https://doi.org/10.5281/zenodo.17020134>.

Table S1 Oligonucleotides for site-directed mutagenesis of pET15b-CtTrl1.

Name	Sequence 5'→3'	Source
CtTrl1-I625Stop_sdm_f	CCGGCACATTtaaCCTGATCGTTC	Sigma
CtTrl1-I625Stop_sdm_r	AGATCTGGCTTGTAGC	Sigma
CtTrl1-D449N_sdm-f	CATCCAAAATaACAACATTACTGGCAGC	Sigma
CtTrl1-D449N_sdm-r	TGCCCCCATCCAAACAGC	Sigma

Table S2 RNA substrates.

Name	Sequence 5'→3'	Source
ASL-4U	AUCUGGAUUUUGCCAGACUGAAG[Phos]	Sigma

Table S3 Oligonucleotides for yeast plasmid and strain construction.

Name	Sequence 5'→3'	Source
Trl1-prom-M_rev	CATCGCTTCTTCGTATGAATAC	Sigma
Trl1-KIN-R389_fwd	CGCACTAAATTTTTAATCTTTCCAATATCAGT	Sigma
pRS-3xHA-fwd	GCGGCCGTTTACCCATACGATGTTC	Sigma
pRS-scTrl1-LIG-End-rev	TTCGTCTACTTTATCATAATCAATGGCGTTTTTT AATTCC	Sigma
Assembly_fragment1_fwd	CTAGAACTAGTGGATCCCCCAAAGGTTCAAGGA ATGCCATGC	Sigma
Assembly_fragment3_rev	TATCGATAAGCTTGATATCGTAGAACGAGAATT TCGACCGAAGGTG	Sigma
pRS306N-K+C_fwd	TCGTACGCTGCAGGTCGACGAAAGGTTCAAGG AATGCC	Sigma
pRS306N-K+C_rev	CGCGCCTTAATTAACCCGGGTAGAACGAGAATT TCGAC	Sigma
Janke_Tr11-LIG-Stop_1	GTTGGAATTAAAAAACGCCATTGATTATGATAAA GTAGACGAATAAAAAGTGGAACGATCATT	Sigma
Trl1_Janke_S2	GGTTAAACGAAATTAACGTTTTTAAGACAGCCA GACCGCGGTCTAATCGATGAATTCGAGCTCG	Sigma

Table S4 Yeast plasmids.

Name	Marker	Source
pRS313-scTrl1-3xHA	His	Köhler <i>et al.</i> , 2025
pRS313-scTrl1-KIN-CPD-3xHA	His	This study
pRS313-scTrl1-LIG-3xHA	His	This study
pRS314	Trp	Sikorski <i>et al.</i> , 1989
pRS314-scTrl1-LIG-3xHA	Trp	This study
pRS306N	<i>natNT2</i>	This study
pRS306N-scTrl1-KIN-CPD	<i>natNT2</i>	This study
pYM18	<i>kanMX4</i>	Janke <i>et al.</i> , 2004
pRS313-scTrl1-D545K-3xHA	His	This study
pRS313-scTrl1-D545K-K579E-3xHA	His	This study
pRS313-scTrl1-D545K-K579E-E587K-3xHA	His	This study

Table S5 Yeast strains.

Yeast strain	Marker	Source
WT, W303 (<i>MATa leu2-3,112 TRP1 can1-100 ura3-1 ADE2 his3-11,15</i>)	-	Peschek and Walter, 2019
<i>trl1</i> -KIN-CPD in <i>ura3</i> (<i>MATa leu2-3,112 TRP1 can1-100 ura3-1::trl1-M1,R389-F827,3xHA,hphNT1 ADE2 his3-11,15</i>)	<i>natNT2</i>	This study
<i>trl1</i> -LIG-E408Stop, <i>trl1</i> -KIN-CPD in <i>ura3</i> (<i>MATa leu2-3,112 TRP1 can1-100 ura3-1::trl1-M1,R389-F827,3xHA,hphNT1 ADE2 his3-11,15 trl1-E408Stop,kanmx</i>)	<i>natNT2, kanMX4</i>	This study
$\Delta trl1$ + p416-ADH-ctTrl1	Ura	Peschek and Walter, 2019
$\Delta trl1$ + pRS313-scTrl1-3xHA	His	Köhler <i>et al.</i> , 2025
$\Delta trl1$ + pRS313-scTrl1-KIN-CPD-3xHA + pRS314-scTrl1-LIG-3xHA	His, Trp	This study
$\Delta trl1$ + pRS313-scTrl1-D545K-3xHA	His	This study
$\Delta trl1$ + pRS313-scTrl1-D545K-K579E-3xHA	His	This study