SUPPLEMENTARY INFORMATION

The zinc-finger protein Red1 orchestrates MTREC submodules and binds the Mtl1 helicase arch domain

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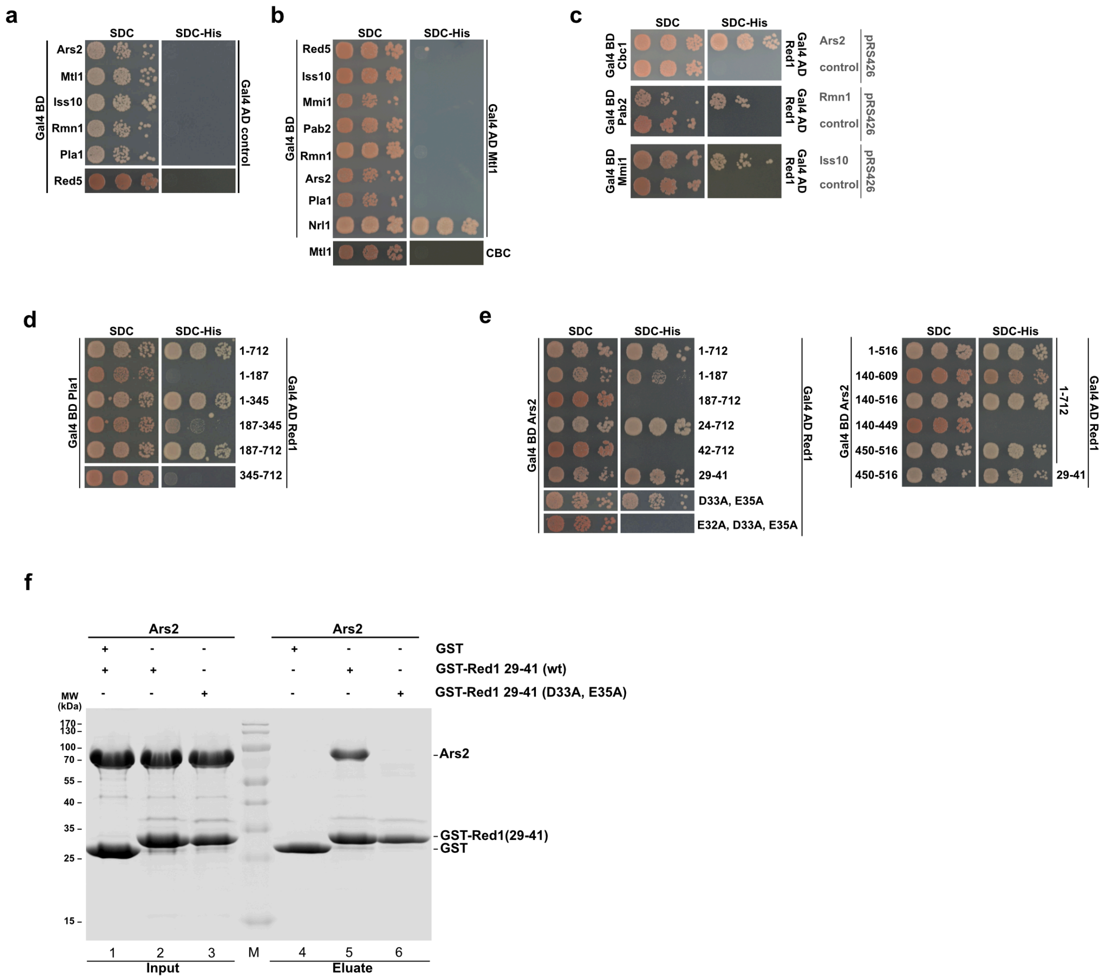
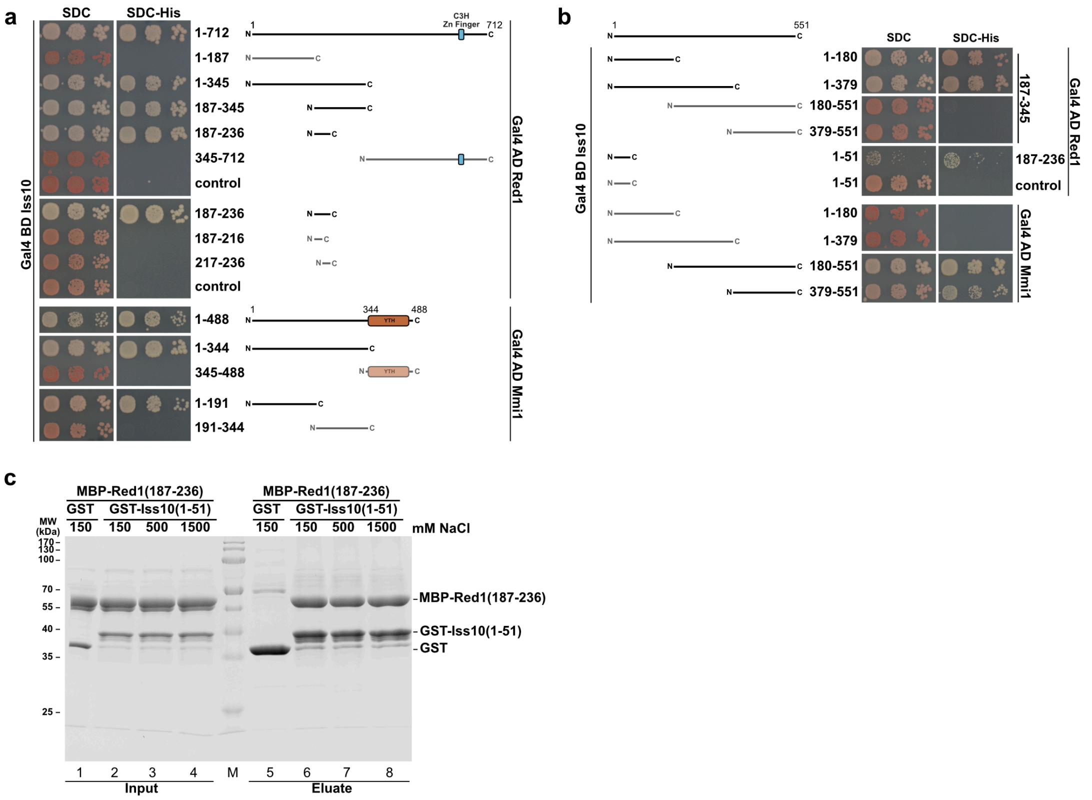


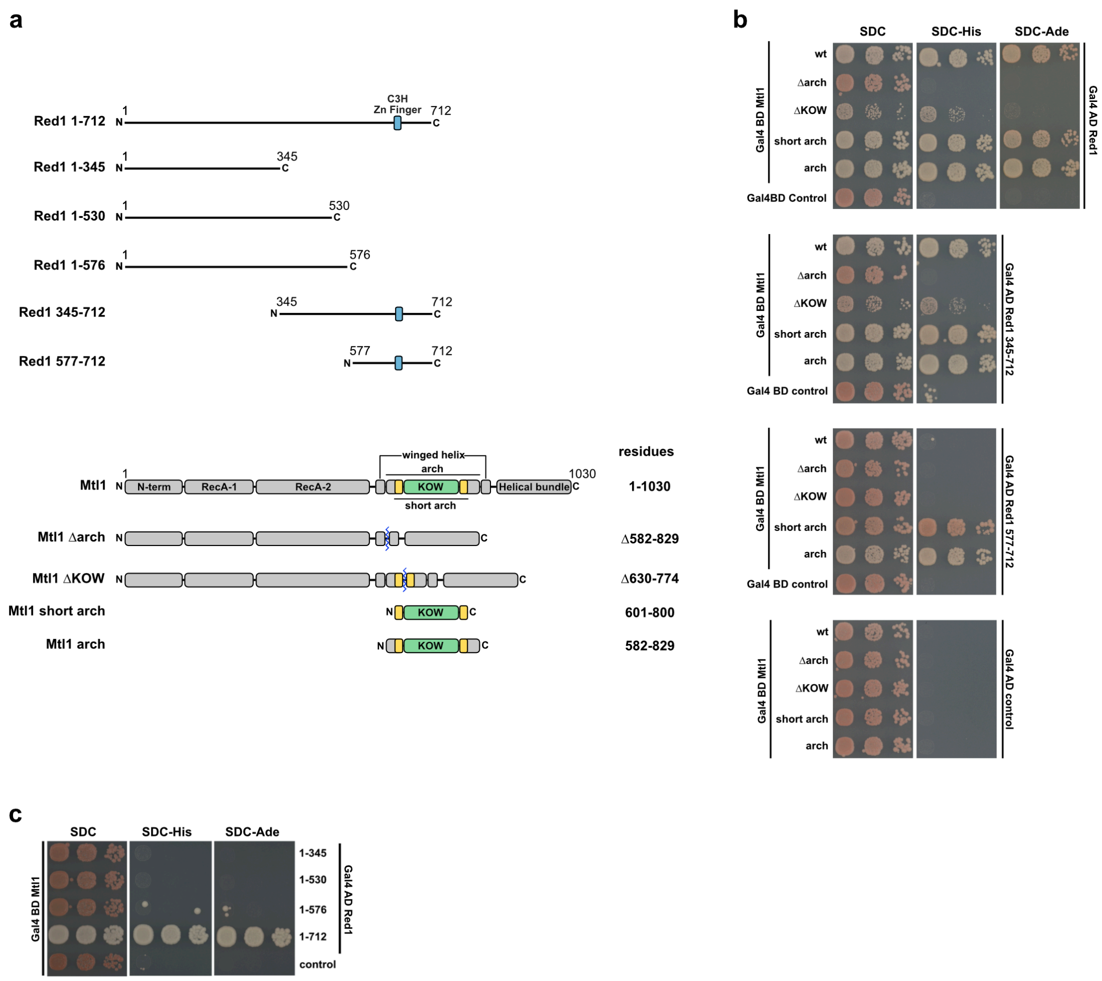
Supplementary Figure 1 Analysis of interactions between MTREC components

a Auto-activation controls for the interactions presented in Fig. 1b. **b** Y2H experiments showing no interaction between Mtl1 and the other MTREC subunits. Nrl1 is used as positive control. Nrl1 interacts with Mtl1 and Ctr1 to form the CNM complex[1,2]. **c** Ars2 bridges Cbc1 and Red1 in Y3H analysis. Similarly, Pab2 and Mmi1 interact with Red1 in presence of Rmn1 and Iss10, respectively. **d** Pla1 interacts with various Red1 truncation variants**. e** Ars2 interacts with various Red1 truncation variants (left panel), and Ars2 truncation variants with Red1 (right panel)**. f** Coomassie stained SDS-PAGE of an *in vitro* GST pull-down assay with the Red1 peptide (residues 29 to 41) and Ars2. The Red1 double mutant (D33A, E35A) does not interact with Ars2.



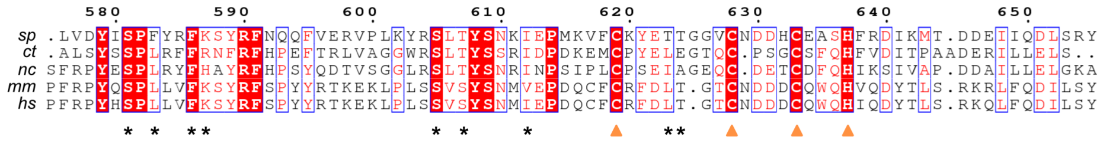
Supplementary Figure 2 Analysis of interactions between the Mmi1-Iss10 submodule and Red1

a, b Y2H analysis of the Mmi1-Iss10 and Red1 interaction. Using Red1, Iss10 and Mmi1 truncation variants, the interacting regions were narrowed down to Iss10 (residues 379 to 551) and Mmi1 (residues 1 to 191) in the Mmi1-Iss10 submodule, and to Red1 (residues 187 to 236) and Iss10 (residues 1 to 51). (c) Coomassie stained SDS-PAGE of an *in vitro* GST pull-down assay with MBP-Red1 (residues 187 to 236) and GST-Iss10 (residues 1 to 51). The Iss10 N-terminal region interacts with the Red1 truncation variant, and this interaction is stable under high salt conditions (1.5 M NaCl).



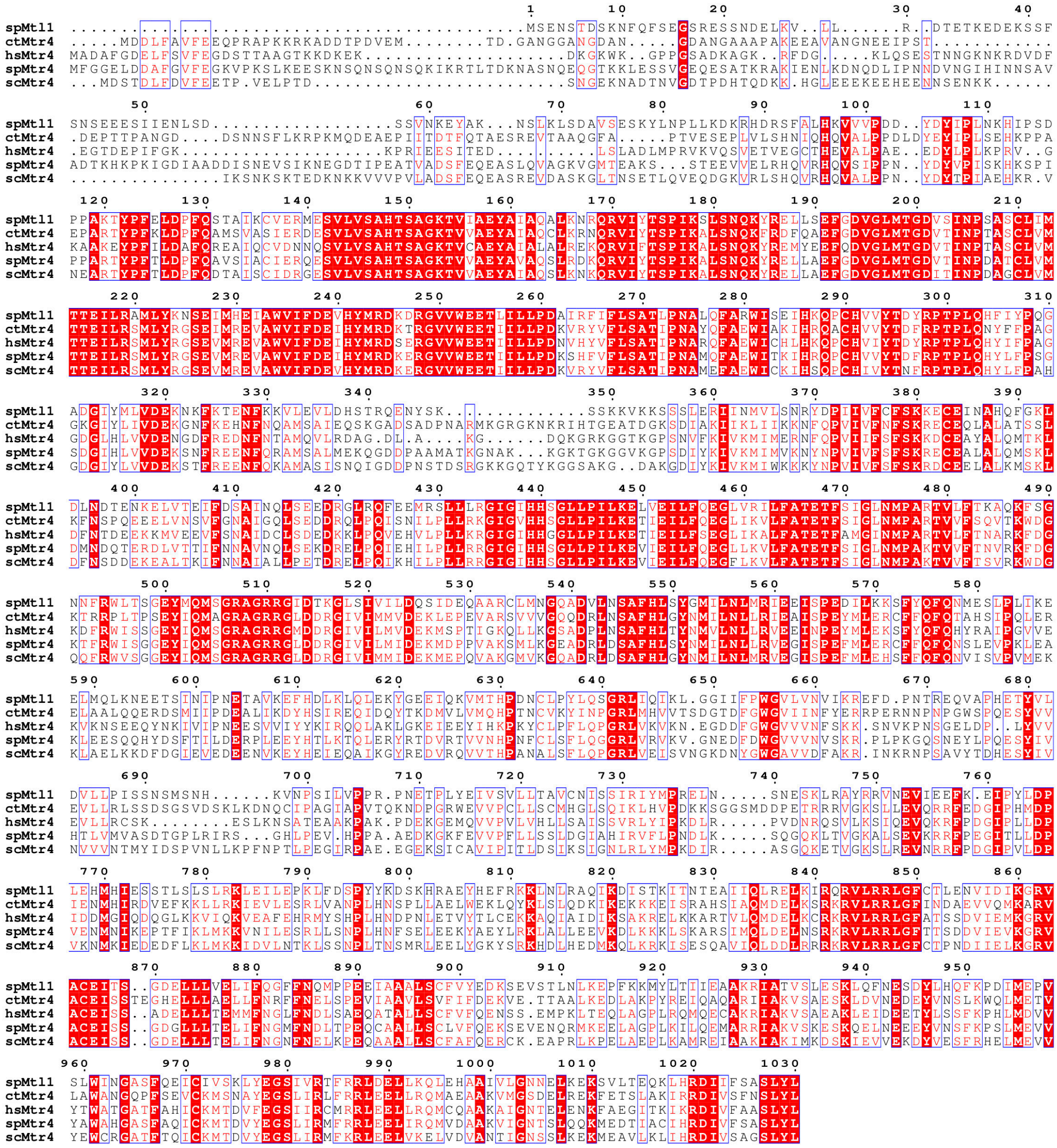
Supplementary Figure 3 Analysis of interactions between Red1 and Mtl1

a Scheme of Red1 and Mtl1 variants used in Y2H analyses. **b** Y2H analysis of Mtl1 and Red1 truncation variants. The Mtl1 ΔKOW domain shows much weaker interaction with Red1 compared to the arch domain indicated by slow growth on SDC-His and no growth on SDC-Ade. **c** Y2H analysis of Mtl1 and Red1 N-terminal truncation variants. Gal4 BD, DNA binding domain; Gal4 AD, activation domain; SDC-Leu-Trp (SDC), SDC-Leu-Trp-His (SDC-His) and SDC-Leu-Trp-Ade(SDC-Ade).



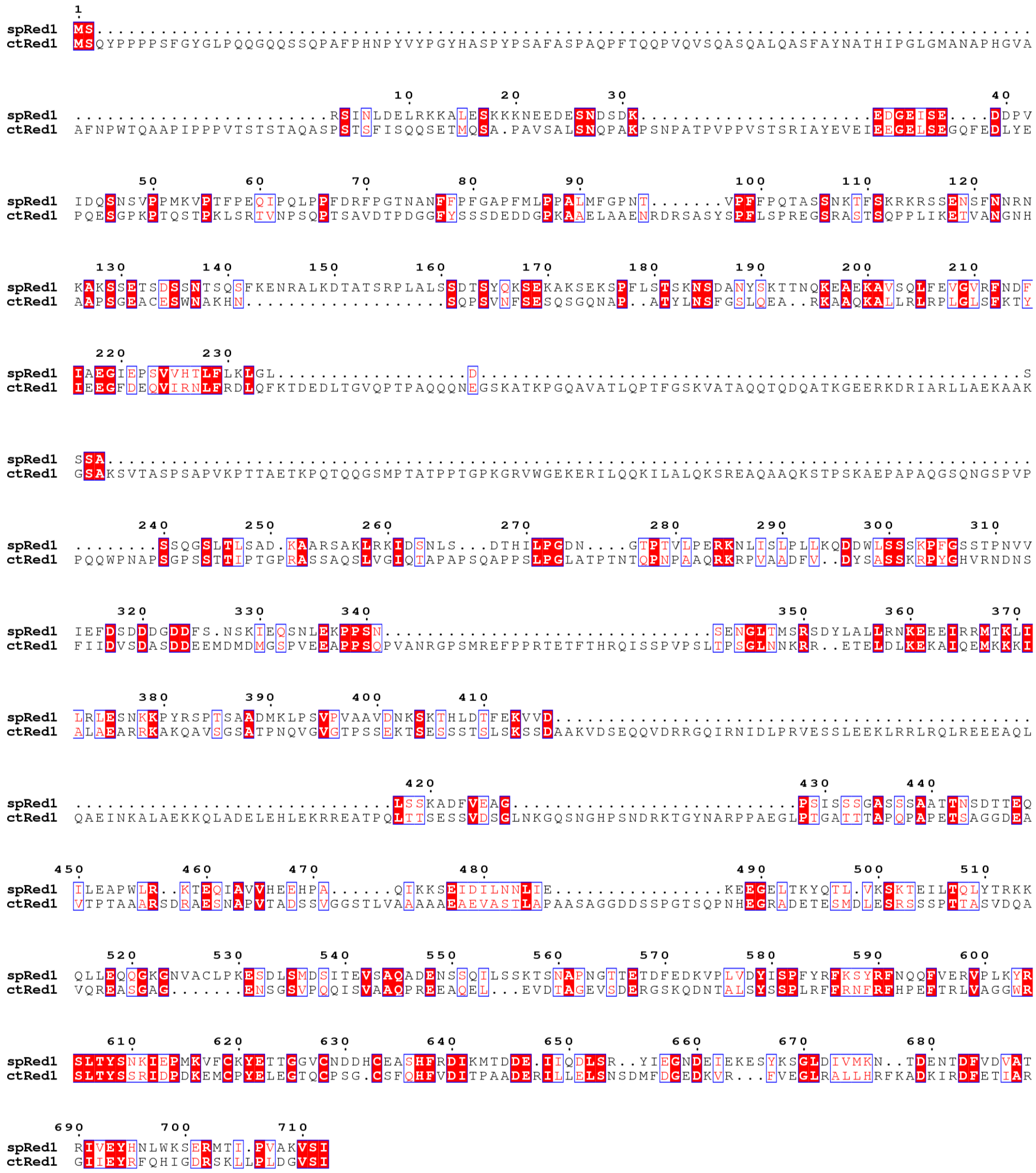
Supplementary Figure 4 Multiple sequence alignment of the conserved Red1 C-terminal region

Sequences from *Chaetomium thermophilum* (*ct)* – *ct*Red1 (Uniprot ID: G0S1V1, see Supplementary Figure 12), *Schizosaccharomyces pombe* (*sp*) – *sp*Red1 (Uniprot ID: Q9UTR8), *Neurospora crassa* (*nc*) – *nc*Red1 (Uniprot ID: V5IR63), *Mus musculus* (*mm*) – *mmZfc3h1* (Uniprot ID: B2RT41) and *Homo sapiens* (*hs*) - *hsZFC3H1 (Uniprot ID: O60293)*. Fully conserved residues are highlighted in red. Residues selected for mutational analysis are marked with an asterisk. Orange arrowheads indicate residues involved in Zinc-finger formation.



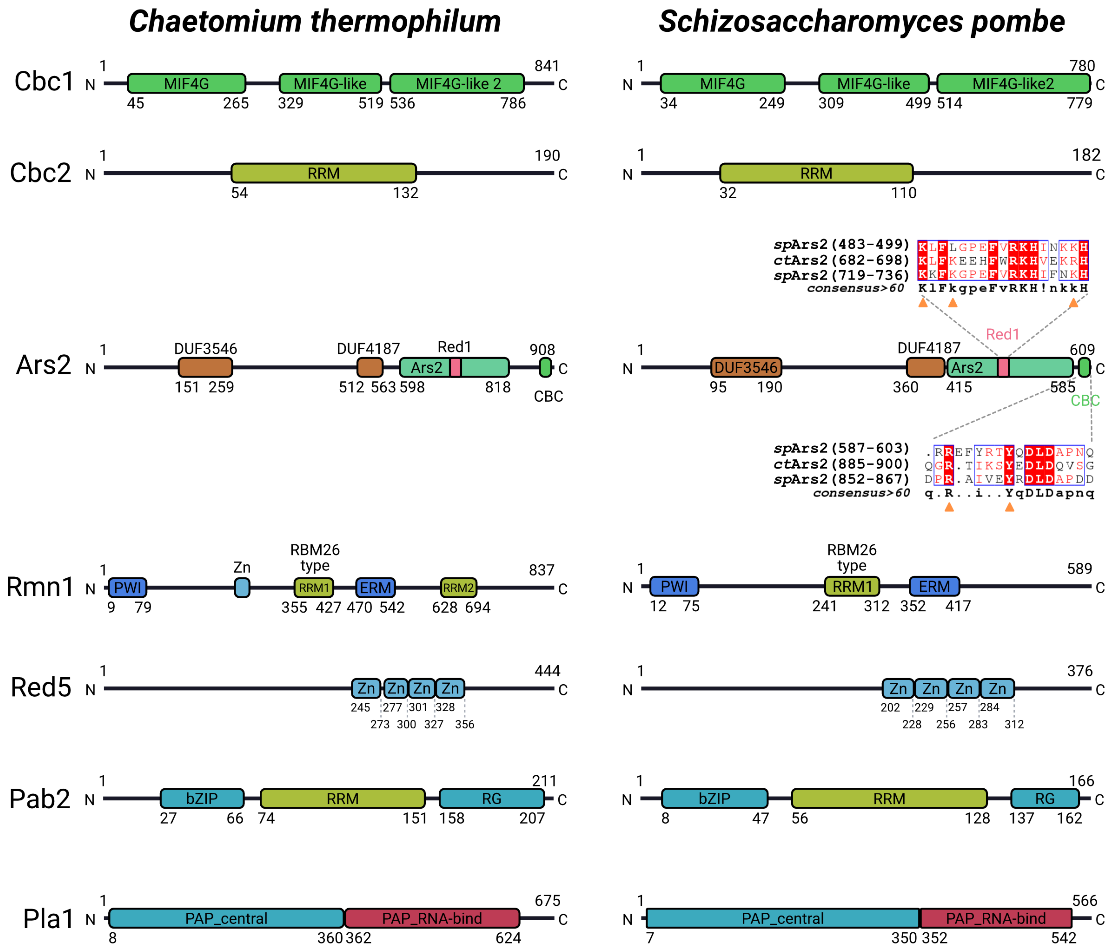
Supplementary Figure 5 Sequence alignment of Mtr4/Mtl1

Multiple sequence alignment of *Chaetomium thermophilum* Mtr4 (*ct*Mtr4, Uniprot ID: G0RZ64) with corresponding region of *Schizosaccharomyces pombe* Mtl1 (*sp*Mtl1*, Uniprot ID:* O13799), *Homo sapiens* Mtr4 (*hs*Mtr4*, Uniprot ID:* P42285), *Schizosaccharomyces pombe* Mtr4 (*sp*Mtr4*, Uniprot ID:* O14232) and *Saccharomyces cerevisiae* Mtr4 (*sc*Mtr4, Uniprot ID: P47047). Fully conserved residues are highlighted in red.



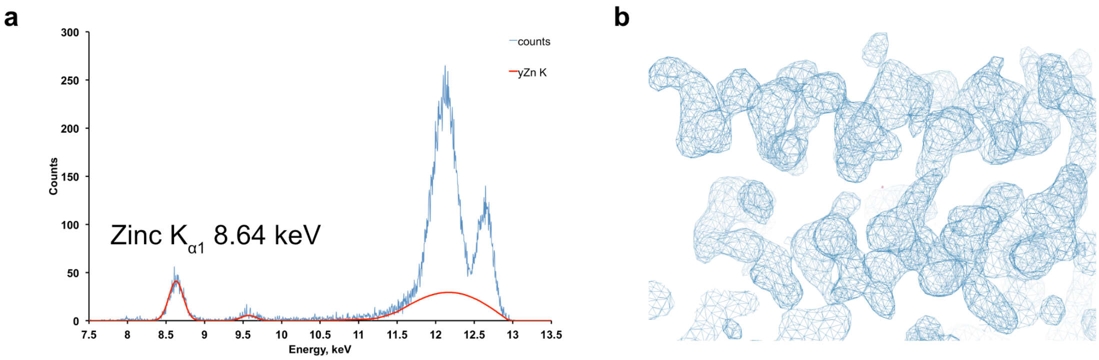
Supplementary Figure 6 Sequence alignment of Red1 from *Chaetomium thermophilum* and *Schizosaccharomyces pombe*

Sequence alignment of *ct*Red1(Uniprot ID: G0S1V1, see Supplement figure 12) and *sp*Red1 (Uniprot ID: Q9UTR8).



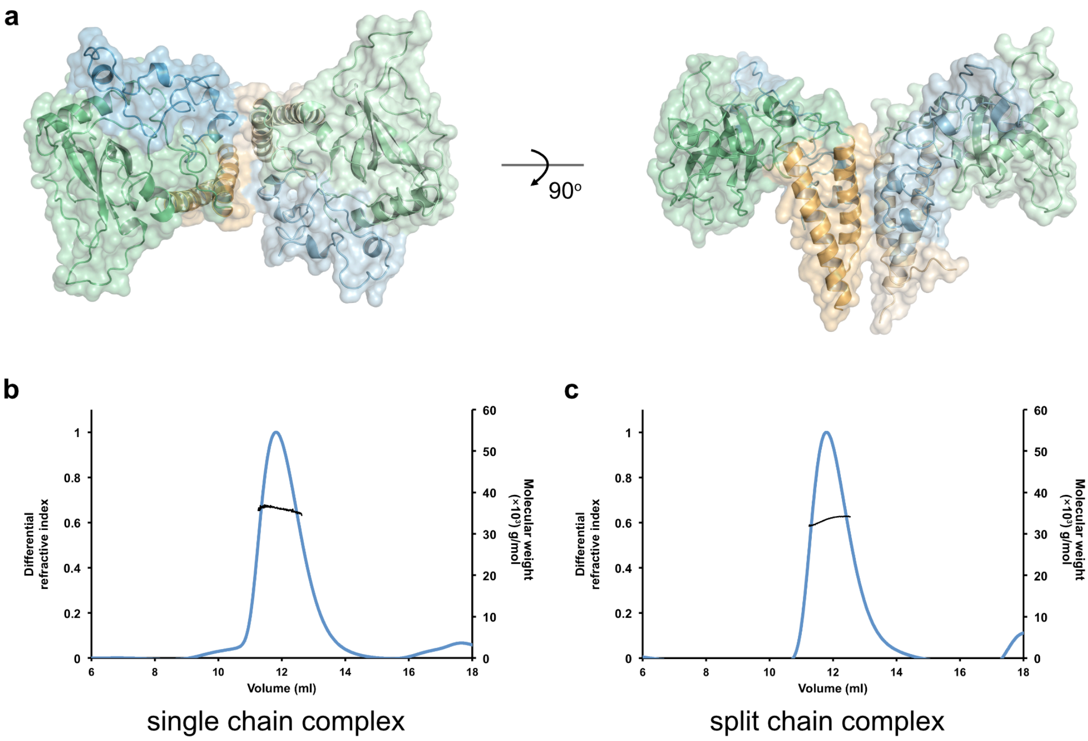
Supplementary Figure 7 Domain organization of MTREC components in of *Chaetomium thermophilum* and *Schizosaccharomyces pombe*

The domain organization of MTREC proteins of *Chaetomium thermophilum* and *Schizosaccharomyces pombe* is shown. *ct*Cbc1 (Uniprot ID: G0SE05) and *sp*Cbc1 (Uniprot ID: O14253); *ct*Cbc2 (Uniprot ID: G0S4F4) and *sp*Cbc2 (Uniprot ID: Q9P383); *ct*Ars2 (Uniprot ID: G0SBQ9) and *sp*Ars2 (Uniprot ID: O94326) – partial alignments of the Red1 and CBC interaction regions are shown. Orange arrowheads indicate residues previously shown to be critical for binding FLASH (PMID: 29703953) and CBC (PMID: 29101316), respectively. *Hs*Ars2 (Uniprot ID: Q9BXP5-4) was used for the alignment; *ct*Rmn1 (Uniprot ID: G0S5V0) and *sp*Rmn1 (Uniprot ID: Q9USP9); *ct*Red5 (Uniprot ID: G0RZM1) and *sp*Red5 (Uniprot ID: O74823); *ct*Pab2 (Uniprot ID: G0S9J4) and *sp*Pab2 (Uniprot ID: O14327); *ct*Pla1 (Uniprot ID: G0S6X0) and *sp*Pla1 (Uniprot ID: Q10295).



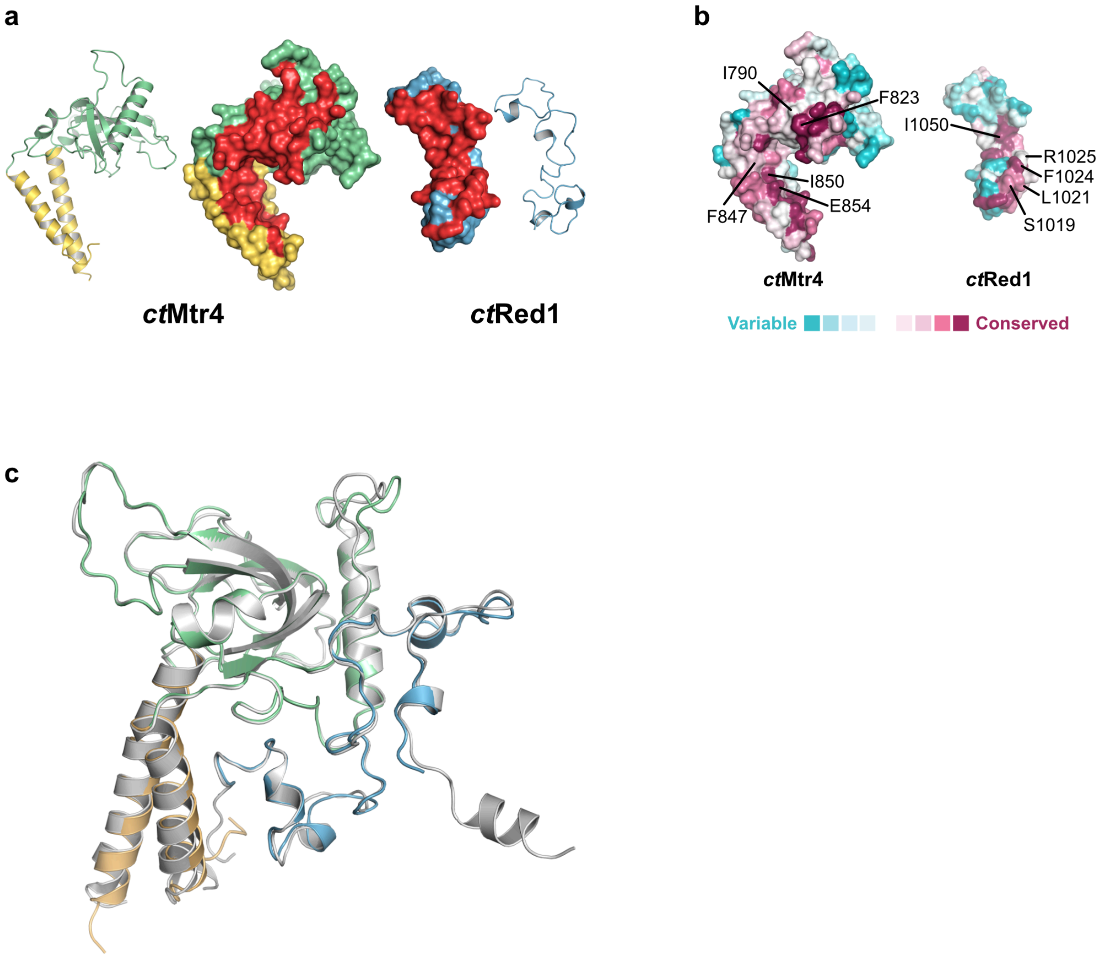
Supplementary Figure 8 X-ray structure determination using Zn-SAD

a X-ray fluorescence (XRF) spectrum revealing the presence of Zn^{2+} in the *ct*Mtr4-*ct*Red1 crystal. **b** Initial composite (2Fo - Fc, contoured at 1.5 σ) electron density map. The figure was made in *Coot*[3].



Supplementary Figure 9 Analysis of the *ct*Mtr4$_{SA}$-*ct*Red1$_{pep}$ complex

a The single-chain *ct*Mtr4$_{SA}$-*ct*Red1$_{pep}$ complex crystallizes with a crystallographic dimer in the ASU. The dimer is shown in cartoon and surface representation. The color scheme is as follows: *ct*Red1$_{pep}$ (blue), *ct*Mtr4 KOW domain (green) and stalk helices (orange). **b, c** SEC-MALS analysis of the oligomerization state for the single-chain complex (in b) and the split-chain complex (in c). Both complexes show a molecular weight of ~34 kDa indicating a monomer in solution.



Supplementary Figure 10 Conservation analysis of the *ct*Mtr4$_{SA}$-*ct*Red1$_{pep}$ interface

a Interaction between *ct*Mtr4$_{SA}$ and *ct*Red1$_{pep}$ involves a large surface area of 1319.8 Å^2 (*ct*Mtr4$_{SA}$) and 1403.2 Å^2 (*ct*Red1$_{pep}$). Both proteins are shown side by side in cartoon and surface representation. Residues involved in the interaction are highlighted (red) in the surface representation. **b** The interacting surfaces of both proteins are shown colored by conservation (analyzed with ConSurf webserver[4]). The majority of residues in the interface are conserved. Variable amino acids are shown in mint-green and conserved amino acids in red. **c** Superposition of single chain (in color) and split chain complex (gray) shows only small differences.

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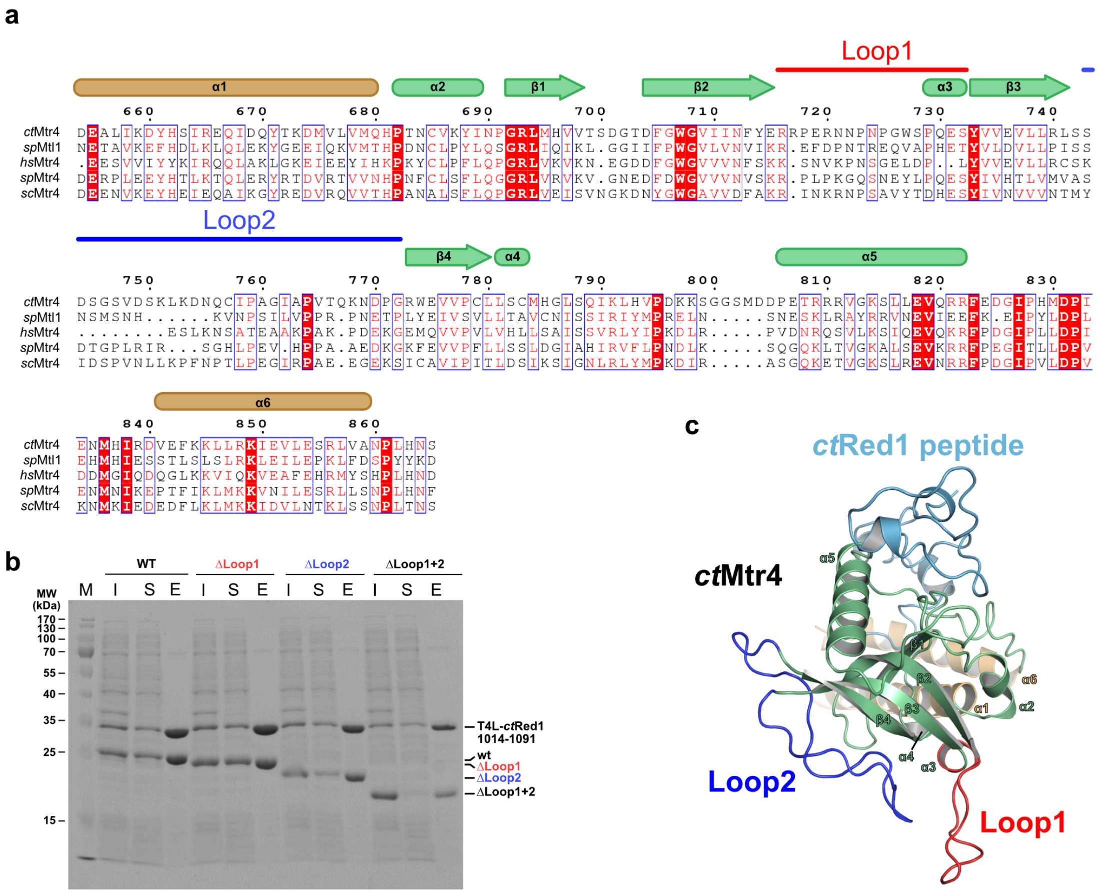
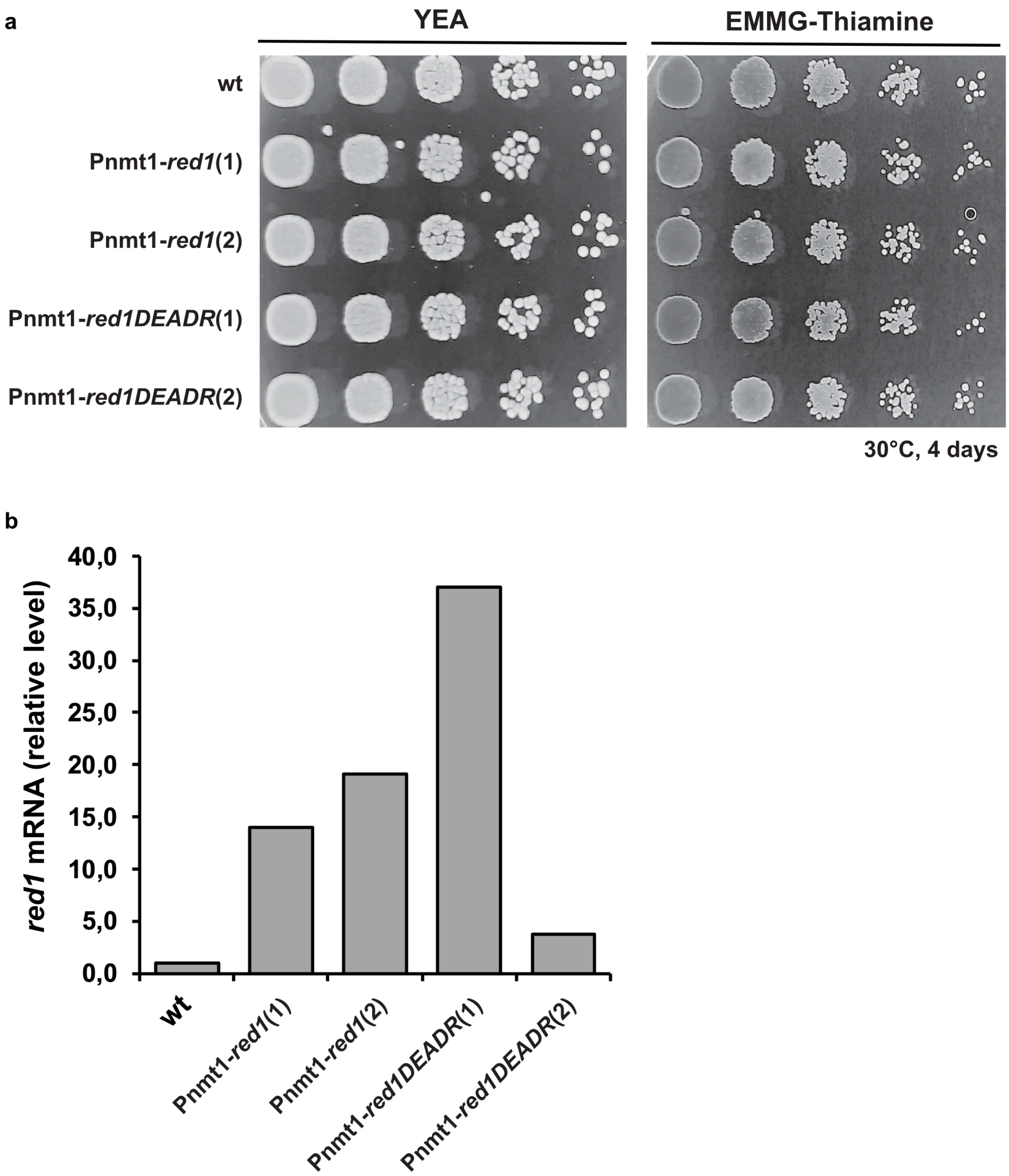
Supplementary Figure 11 PISA analysis of *ct*Mtr4$_{SA}$-*ct*Red1$_{pep}$ interface Analysis of the *ct*Mtr4-*ct*Red1 interaction observed in the crystal structure with the PISA webserver[5] (**HSDC** (Residues making **H**ydrogen/**D**isulphide bond, **S**alt bridge or **C**ovalent); **ASA** Accessible Surface Area, Å²; **BSA** Buried Surface Area, Å². The bars represented the buried area percentage, one bar per 10% ; **ΔiG** Solvation energy effect, kcal/mol.



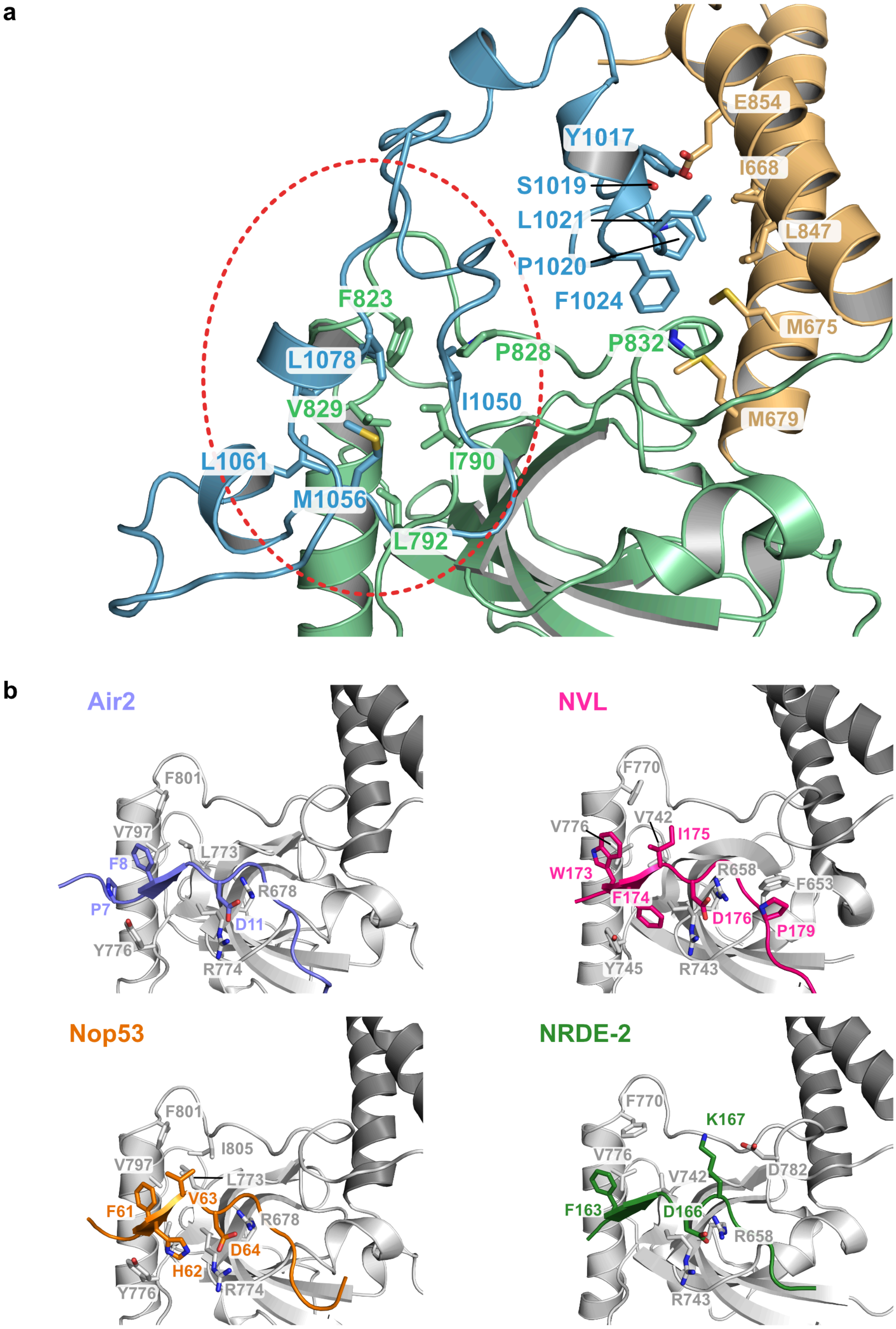
Supplementary Figure 12 Deletion of *ct*Mtr4 KOW domain loops 1 and 2 does not perturb *ct*Red1 binding

a Multiple sequence alignment of *Chaetomium thermophilum* Mtr4$_{SA}$ (*ct*Mtr4, Uniprot ID: G0RZ64) with corresponding region of *Schizosaccharomyces pombe* Mtl1 (*sp*Mtl1*, Uniprot ID:* O13799), *Homo sapiens* Mtr4 (*hs*Mtr4*, Uniprot ID:* P42285), *Schizosaccharomyces pombe* Mtr4 (*sp*Mtr4*, Uniprot ID:* O14232) and *Saccharomyces cerevisiae* Mtr4 (*sc*Mtr4, Uniprot ID: P47047). Fully conserved residues are highlighted in red. The secondary structure is given above the alignment, with stalk helices shown in orange and the KOW domain in green. The KOW domain loops 1 and 2 are highlighted in red and blue. **b** Coomassie stained SDS-PAGE of His$_6$-tagged T4L-*ct*Red1$_{pep}$ co-expressed and purified with untagged *ct*Mtr4$_{SA\Delta loop1}$, *ct*Mtr4$_{SA\Delta loop2}$ and *ct*Mtr4$_{SA\Delta loop1+2}$. Removal of loop1, loop2 or both does not abolish binding to *ct*Red1$_{pep}$. **c** Ribbon representation of the *ct*Mtr4$_{SA}$-*ct*Red1$_{pep}$ structure with loop1 (red) and loop2 (blue) highlighted.



Supplementary Figure 13 ***red1 DEADR*** **mutation is not a dominant negative allele**

Pnmt1-*red1* and Pnmt1-*red1 DEADR* alleles were inserted in the Leu1 locus of wt *S. pombe* genome and (**a**) 5-fold serial dilution of 2 independent strains (labeled with 1 and 2) of each were spotted on YEA media (Pnmt1 promoter is repressed, non-overexpressing condition) and on EMMG-Thiamine media (Pnmt1 promoter is strongly induced, overexpressing condition). (**b**) qPCR analysis of the *red1* mRNA expression level for these strains in EMMG-Thiamine media, compared to *red1* expression in wt *S. pombe* cells. Two biological replicates were performed (labeled with 1 and 2), n = 2. Source data are provided as a Source Data file.



Supplementary Figure 14 Detailed comparison of the *ct*Mtr4-*ct*Red1 crystal structure with known Mtr4 complexes

a Crystal structure of the *ct*Mtr4$_{SA}$-*ct*Red1$_{pep}$ complex (colors are as in Fig. 4). Residues involved in the interaction between *ct*Mtr4-*ct*Red1 are shown in sticks and the binding interface of the AIM or AIM-like adaptor proteins is highlighted by a red dotted line. **b** The structures of Mtr4-Air2 (PDB: 4U4C[6] top left), Mtr4-NVL (PDB: 6RO1[7] top right), Mtr4-Nop53 (PDB: 5OOQ[8] bottom left) and hMTR4-NRDE-2 (PDB: 6IEH[9] bottom right), are shown (same view as in **a**). The stalk helices are shown in dark grey, the KOW domain is in light grey and the interacting peptides are shown in purple (Air2), magenta (NVL), orange (Nop53) and green (NRDE-2). The residues involved in the interaction are shown as sticks.

```
          980       990      1000      1010      1020      1030
            .         .         .         .         .         .
G0S1V1  QISVAAQPREEAQELEVDTAGEVKVVSRGGVDTDQLTDHQVSDERGSKQDNTALSYSSPL
cDNA    QISVAAQPREEAQELEVDTAGE..................VSDERGSKQDNTALSYSSPL

         1040      1050      1060      1070      1080      1090
            .         .         .         .         .         .
G0S1V1  RFFRNFRFHPEFTRLVAGGWRSLTYSSRIDPDKEMCPYELEGTQCPSGCSFQHFVDITPA
cDNA    RFFRNFRFHPEFTRLVAGGWRSLTYSSRIDPDKEMCPYELEGTQCPSGCSFQHFVDITPA

          1100      1110      1120      1130      1140      1150
             .         .         .         .         .         .
G0S1V1  .DERILLELSNSDMFDGEDKVRFVEGLRALLHRFKADKIRDFETIARGIIEYRFQHIGDR
cDNA    ADERILLELSNSDMFDGEDKVRFVEGLRALLHRFKADKIRDFETIARGIIEYRFQHIGDR

          1160
             .
G0S1V1  SKLLPLDGVSI
cDNA    SKLLPLDGVSI
```

Supplementary Figure 15 Sequence alignment of the conserved Red1 C-terminal region

The *ct*Red1 C-terminal region from the Uniprot ID G0S1V1 sequence is compared with the sequence amplified from cDNA. Differences are highlighted by red boxes, which leads to differences in residue numbers. The sequence of our crystallization construct is indicated in blue. Differences between "observed" protein sequences and those annotated in the *Chaetomium thermophilum* genome database have been noticed in the past years and await correction. The *ct*Red1 sequence adds to this list.

Supplementary Table 1. Yeast strain used in this study

Nr.	Name	Relevant Genotype	Reference
S. pombe			
P344	WT	*h+, leu1-32, ura4D18, ade6-M210*	V2-33-H12, Bioneer Inc
P918	WT	*h90, leu1-32, ura4D18, ade6-M210*	Sugiyama et al (2011)[10]
P934	red1Δ	*h90, leu1-32, ura4D18, ade6-M210, red1Δ::kanMX6*	Sugiyama et al (2011)[10]
F3091	Mtl1(L784R/E788R) (E1)	*h90, leu1-32, ura4D18, ade6-M210, mtl1-L784R/E788R::natNT2*	this study
F3092	Mtl1(WT) (E2)	*h90, leu1-32, ura4D18, ade6-M210*	this study
F3093	Mtl1(L784R/E788R) (E3)	*h90, leu1-32, ura4D18, ade6-M210, mtl1-L784R/E788R::natNT2*	this study
F3094	Mtl1(L784R/E788R) (B1)	*h90, leu1-32, ura4D18, ade6-M210, mtl1-L784R/E788R::natNT2*	this study
F3095	Mtl1(WT) (B2)	*h90, leu1-32, ura4D18, ade6-M210*	this study
F3096	Mtl1(L784R/E788R) (B3)	*h90, leu1-32, ura4D18, ade6-M210, mtl1-L784R/E788R::natNT2*	this study
NP70	Red1(DEADR) (A1)	*h90, leu1-32, ura4D18, ade6-M210, red1-DEADR::hphNT1*	this study
NP71	Red1(DEADR) (A4)	*h90, leu1-32, ura4D18, ade6-M210, red1-DEADR::hphNT1*	this study
NP72	Red1(DEADR) (B2)	*h90, leu1-32, ura4D18, ade6-M210, red1-DEADR::hphNT1*	this study
NP73	Red1(WT) (B3)	*h90, leu1-32, ura4D18, ade6-M210*	this study
NP74	Red1(DEADR) (B4)	*h90, leu1-32, ura4D18, ade6-M210, red1-DEADR::hphNT1*	this study

NP75	Red1(DEADR) (D1)	*h90, leu1-32, ura4D18, ade6-M210, red1-DEADR::hphNT1*	this study
NP76	Red1(DEADR) (D2)	*h90, leu1-32, ura4D18, ade6-M210, red1-DEADR::hphNT1*	this study
NP77	Red1(WT) (B3)	*h90, leu1-32, ura4D18, ade6-M210*	this study
NP78	red1Δ (B4)	*h90, leu1-32, ura4D18, ade6-M210, red1Δ::kanMX6*	this study
NP79	red1Δ (E4)	*h90, leu1-32, ura4D18, ade6-M210, red1Δ::kanMX6*	this study
F3321	Pnmt1-Red1 (1)	*h+, HIS, leu1-32, ade6-210, ura4-D18, 5'Leu1-pnmt1-red1-3'Leu1::kanMX6*	this study
F3320	Pnmt1-Red1 (2)	*h+, HIS, leu1-32, ade6-210, ura4-D18, 5'Leu1-pnmt1-red1-3'Leu1::kanMX6*	this study
F3322	Pnmt1-Red1DEADR (1)	*h+, HIS, leu1-32, ade6-210, ura4-D18, 5'Leu1-pnmt1-red1-DEADR-3'Leu1::kanMX6*	this study
F3323	Pnmt1-Red1DEADR (2)	*h+, HIS, leu1-32, ade6-210, ura4-D18, 5'Leu1-pnmt1-red1-DEADR-3'Leu1::kanMX6*	this study
S. cerevisiae			
S145	PJ69-4A	MATα, trp1-901, leu2-3,112, ura3-52, his3-200, gal4Δ, gal80Δ, LYS2::GAL1-HIS3, GAL2-ADE2, met2::GAL7-lacZ	James et al., 1996[11]

Supplementary Table 2. Primers used in this study

Nr.	Name	Sequence (5'->3')
N1	*sp*Cbc1_FW_NcoI	CACCATCACCATCACCCCATGG CTTCTTATCGGGGATCAA
N2	*sp*Cbc1_RE_EcoRI	CGACGGAGCTCGAATTCTTATTT TTCCTCATTAGTTGCATCAG
N3	*sp*Cbc2_FW_NcoI	CACCATCACCATCACCCCATGG CATCAATAACAAGGTTAG
N4	*sp*Cbc2_RE_EcoRI	CGACGGAGCTCGAATTCTTAAT TTTTTTTCCAACGATTGTATC
N13	*ct*Mtr4_FW_NcoI	GCTACCATGGATGACCTCTTTG CCGTC
N14	*ct*Mtr4_RE_BamHI	GCTAGGATCCTTACAGATACAA GCTGTTGAACGAG
N59	*sp*Ars2_215aa_RE	AATTGGTACCTTAGGATCCGTCT TGGTTCCATTTGGAAAGTTG
N60	*sp*Ars2_540aa_RE	AATTGGTACCTTAGGATCCTAAA ATCTGTAAGGATACATACGACG TG
N209	*sp*Red1_345aa_NcoI_FW	TATACCATGGGTCTTACTATGTC TCGTTCAGAT
N210	*sp*Mtl1-dKOW-FW-Frg1-StuI	CTTTCAGAAGAAGATAGAGGCC TC
N211	*sp*Mtl1-dKOW-RE-Frg1-GA	GAACCAGAAGCTGATCCAGAAC CGGTCATTACCTTTTGAATTTCT TCCC
N212	*sp*Mtl1-dKOW-FW-Frg2-GA	TTCTGGATCAGCTTCTGGTTCA GGTTCCTCTACGTTAAGTTTATC TCTTCGAAAAC
N213	*sp*Mtl1-dKOW-RE-Frg2-SacII	GTGTTGCGCAAGAGCCGCGG
N214	*sp*Mtl1-dArch-RE-Frg1-GA	CTCGAAGTTGTATAATCGCCTCA GATTCCATATTTTGGAACTGATA AAAG
N215	*sp*Mtl1-dArch-FW-Frg2-GA	GAGGCGATTATACAACTTCGAG AAC
N241	*sp*Mtl1-dArch-seq1-FW	TTAGACAAAGGGTTTTACGGCG
N287	*sp*Ars2_140aa_FW-NcoI	TATACCATGGTGGACGAGTCAC AAGGAAGAG
N288	*sp*Ars2_516aa_RE-BamHI	TATACTCGAGTTAGGATCCATAT CCATAGAGACATATAGCCACC
N295	*ct*Red1_1014aa_NcoI_FW	TATACCATGGCCTTGTCTTACTC CAGCCCTC
N298	*sp*Red1_577aa_NcoI_FW	TATACCATGGTCGATTATATCTC TCCATTTTACCGC
N299	*sp*Red1_712aa_BamHI-NotI-RE	TATAGCGGCCGCTTAGGATCCA ATGGAAACCTTTGCAACAGGAA TTGTC
N320	*ct*Red1-1030aa-NcoI-FW	TATACCATGGCTCCAGAGTTCA CGCGGCTCG

N321	*ct*Red1-1040aa-NcoI-FW	TATACCATGGGTTGGAGGTCTCTGACTTACAG
N322	*ct*Red1-1091aa-XhoI-RE	TATACTCGAGTTAGGACAGTTCCAGGAGGATCCTTTC
N326	*sp*Mtl1-KOW-NcoI-FW	TATACCATGGGTCATCCTGATAATTGTTTGCCTTATCTG
N328	*sp*Mtl1-Short-Arch-NcoI-FW	TATACCATGGGTATTCCCAATGAGACAGCTGTCAAG
N329	*sp*Mtl1-Short-Arch-BamHI-RE	TATAGGATCCTTAAGAATCCTTGTAGTAAGGGCTGTC
N330	*sp*Mtl1-Arch-NcoI-FW	TATACCATGGGTTTACCTTTAATTAAAGAAGAACTTATGCAATT
N331	*sp*Mtl1-Arch-BamHI-RE	TATAGGATCCTTAAGTATTTGTAATTTTCGTCGATATATCTTTTATC
N332	*sp*Red1-187aa-NcoI-FW	TATACCATGGCAAATTATTCCAAGACTACTAATCAAAAG
N333	*sp*Red1-187aa-BamHI-RE	TATAGGATCCTTATGCATCAGAATTTTTAGATGTTGAAAG
N334	*sp*Red1-345aa-BamHI-RE	TATAGGATCCTTAACCATTTTCACTATTTGAGGGGGG
N350	*sp*Mtl1-KOW-BamHI-RE	TATAGGATCCTTATTCATTCGAGTTCAACTCTCGTGG
N353	*sp*Iss10-180aa-XhoI-RE	TATACTCGAGTTAAGCTTGATTCCCATACAAAGATAAAG
N354	*sp*Iss10-180aa-NcoI-FW	TATACCATGGCTTTTAATCCTTCTTCAGTTCTTCCTAG
N355	*sp*Iss10-379aa-XhoI-RE	TATACTCGAGTTAAGCAGAAGGACGAGGGTGAG
N356	*sp*Iss10-379aa-NcoI-FW	TATACCATGGCTGTCCTTTTAGATAAACCGGTTTC
N357	*sp*Mmi1-344aa-XhoI-RE	TATACTCGAGTTAATTTTCGTCAAGAACAACTCGGG
N358	*sp*Mmi1-345aa-NcoI-FW	TATACCATGGGAATTTCACACCGCTCCCG
N359	*sp*Rmn1-326aa-XhoI-RE	TATACTCGAGTTATGCAAACTTCTTTGGTCGATTGTG
N360	*sp*Rmn1-326aa-NcoI-FW	TATACCATGGCATCCCATAAATCTCCCACTAC
N361	*sp*Ars2-296aa-XhoI-RE	TATACTCGAGTTAAGATTCAATAGTAAAATTTGAATCCAATTG
N362	*sp*Ars2-297aa-NcoI-FW	TATACCATGGAAAATCCAAAAATTCCTACTCACACG
N363	*sp*Ars2-449aa-XhoI-RE	TATACTCGAGTTATTTTGCTTTAATATCCACATTTTCAGG
N364	*sp*Ars2-450aa-NcoI-FW	TATACCATGGGGGCATTACCCGTTGAAGAT
N377	*sp*Red1-236aa-BamHI-RE	TATAGGATCCTTAGGAATCCAGTCCGAGCTTTAAAAA
N378	*sp*Red1-23aa-NcoI-FW	TATACCATGGATGAGAGTAATGACTCTGACAAAG

N379	*sp*Red1-42aa-NcoI-FW	TATACCATGGTTATAGATCAATCAAATTCAGTTCCG
N408	5UTR-*sp*Red1-FW-GA-HindIII	CGGCCGCCAGCTGAAGCTTTACTAAATTACTGACTAGTCCATTAAAAGC
N409	5UTR-*sp*Red1-RE-GA	TTATTTACAGCTAAAACTGGGTTAGTAGTG
N410	*sp*Red1-CDS-FW-GA	AACCCAGTTTTAGCTGTAAATAAATGTCCCGGTCAATAAATCTCGATGAG
N411	*sp*Red1-CDS-RE-GA-AscI	GGCAAGCTAAACAGATCTGGCGCGCCTTAGGATCCAATGGAAACCTTTGCAACAGGAATTG
N412	3UTR-*sp*Red1-FW-GA-SacI	CGCTCGAAGGCTTTAACGAGCTCATTGAAACTGCCAAGCAACATCAAAC
N413	3UTR-*sp*Red1-RE-GA-SacI	ATCTGATATCATCGATGAATTCGAGCTGTGGTATTAATAGTTTGGCCTTTCATCC
N414	*sp*Red1-29-41aa-FW	CATGTCTGACAAAGAAGATGGCGAAATTAGCGAAGACGATCCTTAAG
N415	*sp*Red1-29-41aa-RE	GATCCTTAAGGATCGTCTTCGCTAATTTCGCCATCTTCTTTGTCAGA
N416	*sp*Red1-29-41aa(D33A-E35A)-FW	CATGTCTGACAAAGAAGCTGGCGCAATTAGCGAAGACGATCCTTAAG
N417	*sp*Red1-29-41aa(D33A-E35A)-RE	GATCCTTAAGGATCGTCTTCGCTAATTGCGCCAGCTTCTTTGTCAGA
N422	*sp*Mmi1-191aa-FW-NcoI	TATACCATGGGAAGTTCTCGTTATGTTGAAGAAGAAG
N423	*sp*Mmi1-191aa-RE-BamHI	TATAGGATCCTTATCCAACAACCACTGGGTCCGC
N424	*sp*Red1-scADH1-term-GA-BamHI-FW	TGTTGCAAAGGTTTCCATTGGATCCTAATCTCGAGGCGAATTTCTTATGATTTA
N425	*sp*Red1-scADH1-term-GA-BamHI-RE	AGATCTGGCGCGCCTTAGTCGATTACAACAGGTGTTGTCC
N426	*sp*Iss10-54aa-NcoI-FW	TATACCATGGGTTCTGTTAAACGGAAAGTCGAAATTGC
N427	*sp*Iss10-250aa-NcoI-FW	TATACCATGGGCCCGAGGGCAACTAACGTTGC
N428	*sp*Iss10-315aa-NcoI-FW	TATACCATGGGTAACGAAGAGCCGTCTGTCATTCTG
N429	*sp*Iss10-250aa-XhoI-RE	TATACTCGAGTTACGGAGGAGAAGGAGCTGACAC
N430	*sp*Iss10-315aa-XhoI-RE	TATACTCGAGTTAGTTTTTTACGGAGACAATTGAGTTTTTTTC

N431	*sp*Red1-537aa-seq-FW	TCAATAACCGAGGTTTCCGC
N432	*sp*Pla1-352aa-NcoI-FW	TATACCATGGACTTTTTTCATCG TTATAAGCATTATC
N433	*sp*Pla1-352aa-XhoI-RE	TATACTCGAGTTAGTCGTGTTTT TGAAACAAAGCTGAC
N452	*sp*Iss10-51aa-RE-XhoI	TATACTCGAGTTAATTTTTTAAA GATTTATGTAATCTATCTCTTAA
N453	*sp*Red1-653aa-RE-BamHI	TATAGGATCCTTATGATAAATCT TGTATAATTTCATCATCCG
N464	*ct*Red1-1091aa-C-6xHis-XhoI	TATACTCGAGTTAGTGATGGTG ATGGTGATGAGAACCGGACAGT TCCAGGAGGATCC
N516	*sp*Red1-216aa-BamHI-RE	TATAGGATCCTTAAATAAAATCG TTAAAGCGCACTCC
N517	*sp*Red1-217-236aa-Adaptor-NcoI-FW	CATGGCTGAGGGCATCGAGCCT TCGGTTGTTCATACTTTATTTTTA AAGCTCGGACTGGATTCCTAAG
N518	*sp*Red1-187-216aa-Adaptor-BamHI-RE	GATCCTTAGGAATCCAGTCCGA GCTTTAAAAATAAAGTATGAACA ACCGAAGGCTCGATGCCCTCAG C
N519	*ct*Red1-1040aa-FW-GA-BamHI	GTTCAACAGCTTGTATCTGGGC GGCTCCGGTTCCGGTTGGAGGT CTCTGACTTAC
N520	*ct*Red1-1091aa-RE-GA-BamHI	TGGTGGTGCTCGAGTTAGGATC CGGACAGTTCCAGGAGAATCCT TTCAT
N521	*sp*Mtr4-NcoI-FW-GA	AGAGGAGGACCTGGGTTCCATG GGTTTTGGTGGTGAGTTAGATG ATGC
N522	*sp*Mtr4-BamHI-RE-GA	CGCGGCCGCTCGAGTTAGGATC CCAAATATAGTGATGCACTAAAA ACAATATC
N539	*sp*Red1-288aa-NcoI-FW	TATACCATGGGCATATCTTTACC ACTTTTGAAGCAG
N540	*sp*Red1-287aa-BamHI-RE	TATAGGATCCTTAAAGATTTTTT CTTTCAGGAAGGAC
N547	*ct*Mtr4-short-Arch-NcoI-FW	TATACCATGGGCTCTGGACCCG ACGAGGCGCTCATCAAG
N550	*ct*Mtr4-short-Arch-BamHI-noStop-RE	TATAGGATCCCGAGTTGTGCAG CGGGTTG
N551	*ct*Mtr4-short-Arch-BamHI-RE	TATAGGATCCTTACGAGTTGTG CAGCGGGTTG
N556	*ct*Red1-1091-GA-RE	AGTCAGTGGTGGTGGTGGTGGT GGGATCCGGACAGTTCCAGGA GAATCCTTTCATC
N557	*ct*Red1-1091-STOP-GA-RE	AGTCAGTGGTGGTGGTGGTGGT GGTTAGGATCCGGACAGTTCCA GGAGAATCCTTTCATC

N558	*ct*Red1-1014-GGSGGS-GA-FW	CCCGCTGCACAACTCGGGTGGTTCTGGCGGTTCCGCCTTGTCTTACTCCAGCCC
N577	*ct*Mtr4-Arch-632-NcoI-FW	TATACCATGGGTAGCATCCCCCAGCTAGAGC
N578	*ct*Mtr4-Arch-897-BamHI-RE	TATATTAGGATCCGGAGTGCGCACGCGAGATC
N579	*ct*Mtr4-KOW-dLoop1-FW-QC	GAGGGTAGCGGTTCTTACGTCGTCGAGGTACTCCTCC
N580	*ct*Mtr4-KOW-dLoop1-RE-QC	ACGTAAGAACCGCTACCCTCGTAGAAGTTGATGATGACGCC
N581	*ct*Mtr4-KOW-dLoop2-FW-QC	GGCAGCGGTAGCGGTTCTGGCCGCTGGGAGGTCGTG
N582	*ct*Mtr4-KOW-dLoop2-RE-QC	AGAACCGCTACCGCTGCCGGATAGGCGGAGGAGTACCTC
N583	*sp*Red1-29-41aa-NoSTOP-FW-XhoI	CATGTCTGACAAAGAAGATGGCGAAATTAGCGAAGACGATCCTC
N584	*sp*Red1-29-41aa-NoSTOP-RE-XhoI	TCGAGAGGATCGTCTTCGCTAATTTCGCCATCTTCTTTGTCAGA
N602	*ct*Red1-F1024R-FW-QC	CCAGCCCTCTACGCAGGTTCCGGAACTTCCGATTC
N603	*ct*Red1-F1024R-RE-QC	GAATCGGAAGTTCCGGAACCTGCGTAGAGGGCTGG
N604	*ct*Red1-I1050R-FW-QC	CAGCAGGAGAGATCCTGACAAAGAGATGTGCC
N605	*ct*Red1-I1050R-RE-QC	GTCAGGATCTCTCCTGCTGCTGTAAGTCAGAGAC
N606	*ct*Red1-S1043R-T1045R-FW-QC	TGGAGGAGACTGAGATACAGCAGCAGGATTGATCCTGAC
N607	*ct*Red1-S1043R-T1045R-FW-QC	CTGTATCTCAGTCTCCTCCAACCACCGGCCACG
N608	*ct*Red1-E1060R-FW-QC	CCCATATAGACTGGAGGGGACACAGTGCC
N609	*ct*Red1-E1060R-RE-QC	CCCCTCCAGTCTATATGGGCACATCTCTTTGTCAGG
N610	*sp*Red1_F586R-FW-QC	ATTTTACCGCAGAAAATCTTATCGATTTAATCAACAATTTGTTGAG
N611	*sp*Red1_F586R-RE-QC	CGATAAGATTTTCTGCGGTAAAATGGAGAGATATAATCGAC
N612	*sp*Red1_S605R_T607R-FW-QC	TATCGGAGGCTTAGGTACAGCAATAAGATAGAGCCGATGAA
N613	*sp*Red1_S605R_T607R-RE-QC	CTGTACCTAAGCCTCCGATATTTCAACGGTACTCGCTC
N614	*sp*Red1_I612R-FW-QC	GCAATAAGAGAGAGCCGATGAAAGTTTTCTGTAAATAC
N615	*sp*Red1_I612R-RE-QC	CATCGGCTCTCTCTTATTGCTGTAGGTAAGCGACCG
N616	*sp*Red1_E622R-FW-QC	TGTAAATACAGAACCACTGGTGGTGTTTGTAATGATG

N617	*sp*Red1_E622R-RE-QC	CACCAGTGGTTCTGTATTTACAG AAAACTTTCATCGGCTC
N618	*sp*Red1_259aa-FW-GA-BamHI	GAAGAACGGCCGAAAGCTGGT GGTTCTGGCGGTTCCCGTAAGA TTGACAGTAATCTTAGTG
N619	*sp*Red1_345aa-RE-GA-BamHI	GTCAGTGGTGGTGGTGGTGGT GGGATCCATTTTCACTATTTGAG GGGG
N620	*sp*Red1_288aa-FW-GA-BamHI	GAAGAACGGCCGAAAGCTGGT GGTTCTGGCGGTTCCATATCTTT ACCACTTTTGAAGCAGG
N621	*sp*Red1_321aa-RE-GA-BamHI	GTCAGTGGTGGTGGTGGTGGT GGGATCCATCATCATCGGAATC AAATTCAATAAC
N624	*sp*Red1-236aa-NoStop-BamHI-RE	TATAGGATCCGGAATCCAGTCC GAGCTTTAAAAA
N625	*sp*Red1-345aa-NoStop-BamHI-RE	TATAGGATCCACCATTTTCACTA TTTGAGGGGGG
N652	*sp*Red1_F586A_K587D_Frg1_RE-GA	GATAAGAATCAGCGCGGTAAAA TGGAGAGATATAATCG
N653	*sp*Red1_F586A_K587D_Frg2_FW-GA	ATTTTACCGCGCTGATTCTTATC GATTTAATCAACAATTTGTTGAG
N654	*sp*Red1_I641R_Frg1_RE-GA	CGTCATTTTTCTGTCGCGAAAAT GCGACGCC
N655	*sp*Red1_I641R_Frg2_FW-GA	ATTTTCGCGACAGAAAAATGAC GGATGATGAAATTATACAAG
N656	*sp*Red1_S581D_F583D_Frg1_RE-GA	GCGGTATTCTGGATCGATATAAT CGACAAGCGGTACC
N657	*sp*Red1_S581D_F583D_Frg2_FW-GA	TATATCGATCCAGAATACCGCG CTGATTCTTATCGAT
N658	*sp*Red1_T623D_T623R_Frg1_RE-GA	CACCACCACGATCTTCGTATTTA CAGAAAACTTTCATCGG
N659	*sp*Red1_T623D_T623R_Frg2_FW-GA	TACGAAGATCGTGGTGGTGTTT GTAATGATGACCATTG
N660	*sp*Red1-576aa-BamHI-STOP-XhoI-RE	ATATCTCGAGTTAGGATCCAAG CGGTACCTTATCCTCAAAATC
N661	*sp*Red1_5AA-mutant_dete*ct*_RE	ATCAGCGCGGTATTCTGGATC
N662	*sp*Red1-602aa-NcoI-FW	TATACCATGGGCAAATATCGGT CGCTTACCTACAGC
N663	*sp*Mlt1-SA-F666E-FW-QC	AAAAGAGAAGAAGATCCAAACA CTCGTGAACAAGTTG
N664	*sp*Mlt1-SA-F666E-RE-QC	AGTGTTTGGATCTTCTTCTCTTT TTATGACATTGACAAGTACAC
N665	*sp*Mlt1-SA-I652E-FW-QC	GGGGTATTGAATTTCCTTGGGG TGTACTTGTCAATG
N666	*sp*Mlt1-SA-I652E-RE-QC	CCAAGGAAATTCAATACCCCCT AATTTTATTTGTATAAGTCG
N683	*sp*Red1_F586A_Frg1_RE-GA	CGATAAGATTTGGCGCGGTAAA ATGGAGAGATATAATC

N684	*sp*Red1_F586A_Frg2_FW-GA	TACCGCGCCAAATCTTATCGATTTAATCAACAATTTGTTG
N685	*sp*Red1_K587D_Frg1_RE-GA	AATCGATAAGAGTCAAAGCGGTAAAATGGAGAGATATAATC
N686	*sp*Red1_K587D_Frg2_FW-GA	ACCGCTTTGACTCTTATCGATTTAATCAACAATTTGTTG
N687	*sp*Red1_F586A_mut_check_RE	GTTGATTAAATCGATAAGATTTGGC
N688	*sp*Red1_F586F_wt_check_RE	ATTGTTGATTAAATCGATAAGATTTAAA
N689	*sp*Red1_K587D_mut_check_RE	ATTGTTGATTAAATCGATAAGAGTC
N690	*sp*Red1_K587K_wt_check_RE	CAAATTGTTGATTAAATCGATAAGATTT
N696	*sp*Mtl1_I730R-frg1-GA-RE	ATAAATACGTCTAGAAGAAATGTTACAAACAG
N697	*sp*Mtl1_I730R-frg2-GA-FW	TAACATTTCTTCTAGACGTATTTATATGCC
N698	*sp*Mtl1_F758R-frg1-GA-RE	GTATTTCCTTTCTTTCTTCTATAACCTCATTTAC
N699	*sp*Mtl1_F758R-frg2-GA-FW	GTTATAGAAGAAAGAAAGGAAATACCTTATTTAG
N700	*sp*Mtl1_L781R-frg1-GA-RE	CAAGTTTTCGTCTAGATAAACTTAACGTAG
N701	*sp*Mtl1_L781R-frg2-GA-FW	TTAAGTTTATCTAGACGAAAACTTGAAATTC
N702	*sp*Mtl1_L784R_E788R-frg1-GA-RE	GGTCTAAGAATTTCTCTTTTTCGAAGAGATAAACTTAACG
N703	*sp*Mtl1_L784R_E788R-frg2-GA-FW	CGAAAAAGAGAAATTCTTAGACCCAAACTCTTCGACAGCC
N704	*sp*Mtl1-dSA-frg1-GA-RE	CCAGAACCTGATCCAGAACCCTCATTGGGAATATTAATTGAAGTC
N705	*sp*Mtl1-dSA-frg2-GA-FW	GTTCTGGATCAGGTTCTGGTTCAGATTCTAAACATAGGGCCGAATATC
N725	*sp*Mtl1_E788R-frg1-GA-RE	GAAATTCTTAGACCCAAACTCTTCGACAGCCC
N726	*sp*Mtl1_E788R-frg2-GA-FW	GAGTTTGGGTCTAAGAATTTCAAGTTTTCGAAGAG
N/A	Red1-qPCR-FW	CGACCATTGGCTTTATCATCC
N/A	Red1-qPCR-RE	TTCAAACAACTGACTCACAGC
N/A	GAPDH-qPCR-FW	AACATCATCCCCTCCTCCAC
N/A	GAPDH-qPCR-RE	GCCTTGATGTCCTCGTAGTTG

Supplementary Table 3. Plasmids used in this study

pND #	Plasmid	Selection	References
***E. coli* expression plasmids**			
72	pET_His-EYFP	Kan	Gunter Stier
76	pET_His_1a-EYFP	Kan	Gunter Stier
81	pET_GST_1a-EYFP	Kan	Gunter Stier
82	pET_MBP_1a-EYFP	Kan	Gunter Stier
92	pET_His-*ct*Mtr4_1-1097aa	Kan	this study
434	pET_GST_1a-Stop-control	Kan	this study
565	pET_GST_1a-*sp*Red1_29-41aa	Kan	this study
566	pET_GST_1a-*sp*Red1_29-41aa-D33A, E35A	Kan	this study
644	pET21d-GST_*a*-*ct*Red1_1040-1091aa	Amp	this study
646	pET21d-ZZ_*a*-*ct*Red1_1040-1091aa	Amp	this study
689	pET21d_MBP-*sp*Iss10_1-51aa	Amp	this study
690	pET_GST_1a-*sp*Iss10_1-51aa	Kan	this study
699	pET_GST_1a-*sp*Red1_1-236aa	Kan	this study
733	pET_MBP_1a-*sp*Red1_187-236aa	Kan	this study
828	pET21d_T4L_xtal-*ct*Red1_1014-1091aa	Amp	this study
865	pET24d-*ct*Mtr4_654-866aa	Kan	this study
868	pET24dC-6xHis-*ct*Mtr4-*ct*Red1_654-865 | 1014-1091aa	Kan	this study
887	pET24d-*ct*Mtr4_654-866aa-dLoop1	Kan	this study
888	pET24d-*ct*Mtr4_654-866aa-dLoop2	Kan	this study
917	pET24d-*ct*Mtr4_654-866aa-dLoop1+2	Kan	this study
919	pET21d-Gb1_*a*-*ct*Red1_1014-1091aa	Amp	this study
936	pET21d_T4L_xtal-*ct*Red1_1014-1091aa-F1024R	Amp	this study
937	pET21d_T4L_xtal-*ct*Red1_1014-1091aa-I1050R	Amp	this study
938	pET21d_T4L_xtal-c*t*Red1_1014-1091aa-S1043R,T1045R	Amp	this study
939	pET21d_T4L_xtal-*ct*Red1_1014-1091aa-E1060R	Amp	this study
1003	pET_His_1a-*ct*Mtr4_short arch	Kan	this study
Y2H/Y3H plasmids			
333	pGBKT7	Kan	this study
334	pGADT7	Amp	this study
367	pGBKT7-*sp*Red1_1-712aa	Kan	this study
368	pGADT7-*sp*Red1_1-712aa	Amp	this study
369	pGBKT7-*sp*Mtl1_1-1030aa	Kan	this study
370	pGADT7-*sp*Mtl1_1-1030aa	Amp	this study
371	pGBKT7-*sp*Red1_345-712aa	Kan	this study
376	pGBKT7-*sp*Ars2_1-609aa	Kan	this study

377	pGBKT7-*ct*Red1_1014-1055aa	Kan	this study
378	pGBKT7-*ct*Red1_1014-1152aa	Kan	this study
379	pGBKT7-*ct*Red1_676-1055aa	Kan	this study
380	pGBKT7-*sp*Red1_577-712aa	Kan	this study
387	pG4ADC111_mod	Amp	Modified from[12]
388	pG4ADHAN111_mod	Amp	Modified from[12]
389	pG4BDN22_mod	Amp	Modified from[12]
390	pG4BDC22_mod	Amp	Modified from[12]
396	pGBKT7-*sp*Mtl1_dArch	Kan	this study
397	pGBKT7-*sp*Mtl1_dKOW	Kan	this study
398	pGADT7-*sp*Red1_345-712aa	Amp	this study
399	pGADT7-*sp*Red1_577-712aa	Amp	this study
400	pRS426	Amp	Tomlin et al., 2011 [13]
411	pGBKT7-*sp*Red5_1-376aa	Kan	this study
412	pGBKT7-*sp*Iss10_1-551aa	Kan	this study
413	pGBKT7-*sp*Mmi1_1-488aa	Kan	this study
414	pGBKT7-*sp*Pab2_1-166aa	Kan	this study
415	pGBKT7-*sp*Rmn1_1-590aa	Kan	this study
420	pG4BDN-*sp*Nrl1_1-972aa	Amp	this study
435	pGADT7-*sp*Red5_1-376aa	Amp	this study
436	pGADT7-*sp*Iss10_1-551aa	Amp	this study
437	pGADT7-*sp*Mmi1_1-488aa	Amp	this study
438	pGADT7-*sp*Pab2_1-166aa	Amp	this study
439	pGADT7-*sp*Rmn1_1-590aa	Amp	this study
444	pGBKT7-*sp*Ars2_140-516aa	Kan	this study
446	pGADT7-*sp*Red1_1-187aa	Amp	this study
447	pGADT7-*sp*Red1_1-345aa	Amp	this study
448	pGADT7-*sp*Red1_187-345aa	Amp	this study
449	pGADT7-*sp*Red1_187-712aa	Amp	this study
450	pGBKT7-*sp*Mtl1_short arch	Kan	this study
451	pGBKT7-*sp*Mtl1_Arch	Kan	this study
452	pGBKT7-*sp*Ars2_1-516aa	Kan	this study
453	pGBKT7-*sp*Ars2_79-609aa	Kan	this study
454	pGBKT7-*sp*Ars2_140-609aa	Kan	this study
469	pGBKT7-*sp*Pla1_1-566aa	Kan	this study
473	pRS426-ADH1	Amp	this study
474	pGBKT7-*sp*Iss10_1-180aa	Kan	this study
475	pGBKT7-*sp*Iss10_1-379aa	Kan	this study
476	pGBKT7-*sp*Iss10_180-379aa	Kan	this study
477	pGBKT7-*sp*Iss10_180-551aa	Kan	this study
478	pGBKT7-*sp*Iss10_379-551aa	Kan	this study
479	pGADT7-*sp*Mmi1_1-344aa	Amp	this study
480	pGADT7-*sp*Mmi1_345-488aa	Amp	this study

481	pGBKT7-*sp*Rmn1_1-326aa	Kan	this study
482	pGBKT7-*sp*Rmn1_326-590aa	Kan	this study
483	pGADT7-*sp*Rmn1_1-326aa	Amp	this study
484	pGADT7-*sp*Rmn1_326-590aa	Amp	this study
485	pGBKT7-*sp*Ars2_140-296aa	Kan	this study
486	pGBKT7-*sp*Ars2_140-449aa	Kan	this study
487	pGBKT7-*sp*Ars2_297-516aa	Kan	this study
488	pGBKT7-*sp*Ars2_450-516aa	Kan	this study
490	pGBKT7-*sp*Pab2_1-136aa	Kan	this study
494	pGADT7-*sp*Red1_187-236aa	Amp	this study
495	pGADT7-*sp*Red1_24-712aa	Amp	this study
496	pGADT7-*sp*Red1_42-712aa	Amp	this study
497	pRS426-ADH1-*sp*Red1_1-712aa	Amp	this study
498	pRS426-ADH1-*sp*Red5_1-376aa	Amp	this study
499	pRS426-ADH1-*sp*Iss10_1-551aa	Amp	this study
500	pRS426-ADH1-*sp*Mmi1_1-488aa	Amp	this study
501	pRS426-ADH1-*sp*Pab2_1-166aa	Amp	this study
502	pRS426-ADH1-*sp*Rmn1_1-590aa	Amp	this study
507	pG4BDN-*sp*Mtl1_1-1030aa	Amp	this study
508	pG4ADHAN-*sp*Mtl1_1-1030aa	Amp	this study
509	pG4ADHAN-*sp*Red1_1-712aa	Amp	this study
526	pG4ADHAN-*sp*Mtl1_dKOW	Amp	this study
527	pG4ADHAN-*sp*Mtl1_dArch	Amp	this study
528	pG4ADHAN-*sp*Mtl1_short arch	Amp	this study
529	pG4ADHAN-*sp*Mtl1_Arch	Amp	this study
542	pGADT7-*sp*Red1_29-41aa	Amp	this study
543	pGADT7-*sp*Red1_29-41aa-D33A, E35A	Amp	this study
548	pGADT7-*sp*Mmi1_1-191aa	Amp	this study
549	pGADT7-*sp*Mmi1_191-344aa	Amp	this study
550	pG4ADHAN-*sp*Mtl1_short arch-dKOW	Amp	this study
551	pG4ADHAN-*sp*Mtl1_Arch-dKOW	Amp	this study
556	pGBKT7-*sp*Iss10_1-250aa	Kan	this study
557	pGBKT7-*sp*Iss10_1-315aa	Kan	this study
558	pGBKT7-*sp*Iss10_54-379aa	Kan	this study
559	pGBKT7-*sp*Iss10_250-551aa	Kan	this study
560	pGBKT7-*sp*Iss10_315-551aa	Kan	this study
561	pGBKT7-*sp*Pla1_1-352aa	Kan	this study
562	pGBKT7-*sp*Pla1_352-566aa	Kan	this study
563	pGBKT7-*sp*Ars2_440-516aa	Kan	this study
574	pG4BDN-*sp*Cbc1_1-780aa	Amp	this study
575	pG4ADHAN-*sp*Cbc1_1-780aa	Amp	this study
576	pRS426-ADH1-*sp*Cbc1_1-780aa	Amp	this study
577	pG4BDN-*sp*Cbc2_1-182aa	Amp	this study

578	pG4ADHAN-*sp*Cbc2_1-182aa	Amp	this study
579	pRS426-ADH1-*sp*Cbc2_1-182aa	Amp	this study
580	pG4BDN-*sp*Ars2_1-609aa	Amp	this study
581	pG4ADHAN-*sp*Ars2_1-609aa	Amp	this study
582	pRS426-ADH1-*sp*Ars2_1-609aa	Amp	this study
589	pG4BDN-*sp*Ntr1_1-797aa	Amp	this study
668	pG4BDN-*sp*Mtl1_short arch	Amp	this study
669	pG4BDN-*sp*Mtl1_Arch	Amp	this study
670	pG4ADC-*sp*Cbc2_1-182aa	Amp	this study
672	pG4BDC-*sp*Cbc2_1-182aa	Amp	this study
714	pGADT7-*sp*Red1_50-345aa	Amp	this study
715	pGADT7-*sp*Red1_100-345aa	Amp	this study
760	pGBKT7-*sp*Iss10_1-51aa	Kan	this study
761	pGADT7-*sp*Red1_187-216aa	Amp	this study
762	pGADT7-*sp*Red1_217-236aa	Amp	this study
776	pG4ADHAN-*sp*Red1_1-236aa	Amp	this study
779	pG4ADHAN-*sp*Red1_240-712aa	Amp	this study
1004	pGADT7-*sp*Red1_1-712aa-I612R	Amp	this study
1016	pGADT7-*sp*Red1_1-712aa-F585A_K586D	Amp	this study
1017	pGADT7-*sp*Red1_1-712aa-I641R	Amp	this study
1018	pGADT7-*sp*Red1_1-712aa-F585A_K586D_I612R	Amp	this study
1019	pGADT7-*sp*Red1_1-712aa-I612R_I641R	Amp	this study
1041	pGADT7-*sp*Red1_1-712aa-S581D_F583E_F586A_R587D_I612R	Amp	this study
1042	pGADT7-*sp*Red1_1-712aa-F586A_R587D_I612R_T623D_T624R	Amp	this study
1044	pG4ADHAN-*sp*Red1_1-530aa	Amp	this study
1045	pG4ADHAN-*sp*Red1_1-576aa	Amp	this study
1046	pG4ADC-*sp*Red1_1-576aa	Amp	this study
1061	pG4BDN-*sp*Mtl1_1-1030aa-I730R	Amp	this study
1062	pG4BDN-*sp*Mtl1_1-1030aa-F758R	Amp	this study
1063	pG4BDN-*sp*Mtl1_1-1030aa-L781R	Amp	this study
1064	pG4BDN-*sp*Mtl1_1-1030aa-L784R_E788R	Amp	this study
1065	pG4BDN-*sp*Mtl1_1-1030aa-dSA	Amp	this study

S. pombe integration plasmids

1039	pFab6-Red1-gDNA_WT	Amp	this study
1057	pFab6-Red1-gDNA_S581D_F583E_F586A_R587D_I612R	Amp	this study
1068	pFab6-Nat2-Mtl1-L784R_E788R	Amp	this study

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