Supplementary Information

Concerted removal of the Erb1-Ytm1 complex in ribosome biogenesis relies on an elaborate interface

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Supplementary Figure 1-11

Supplementary Table 1 & 2

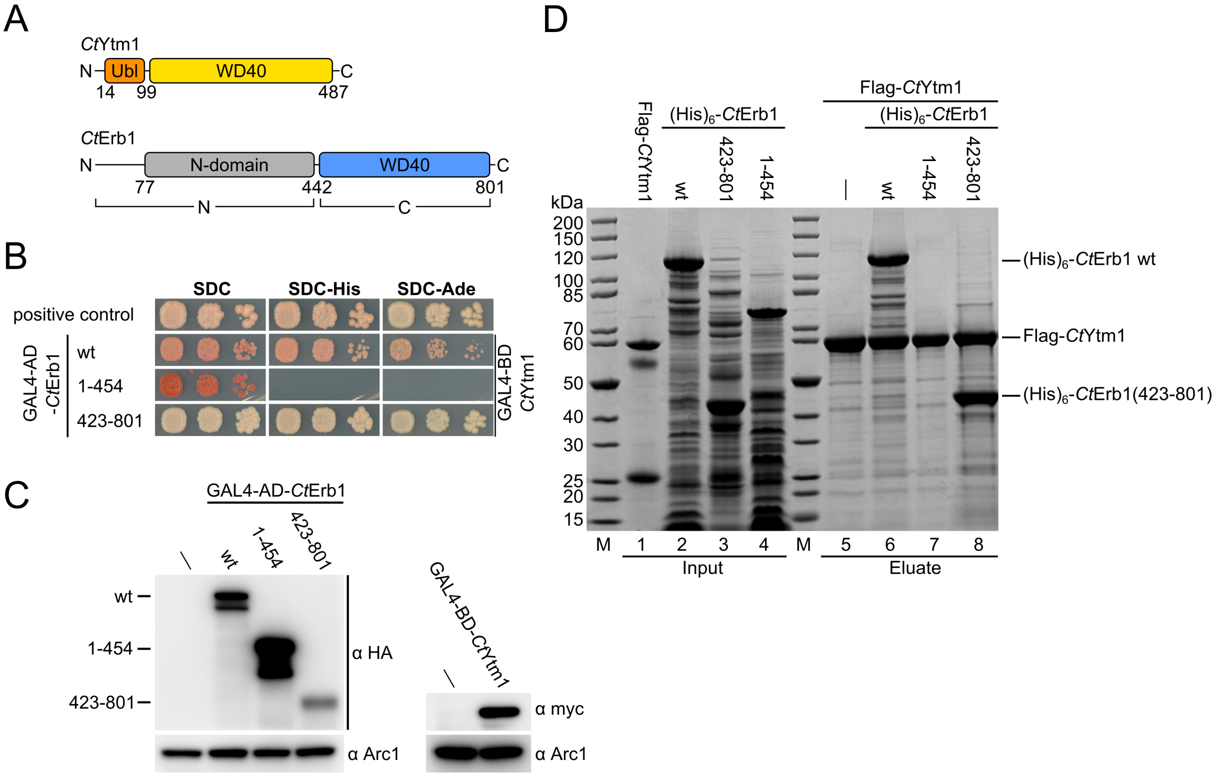
Notes for crystal structure:

The following residues could not be modelled, either due to ambiguous or missing electron density and have been omitted from the final structure:

Erb1, chain A and C: the first 8 residues of the His_6-tag, 422-431 and 551-563

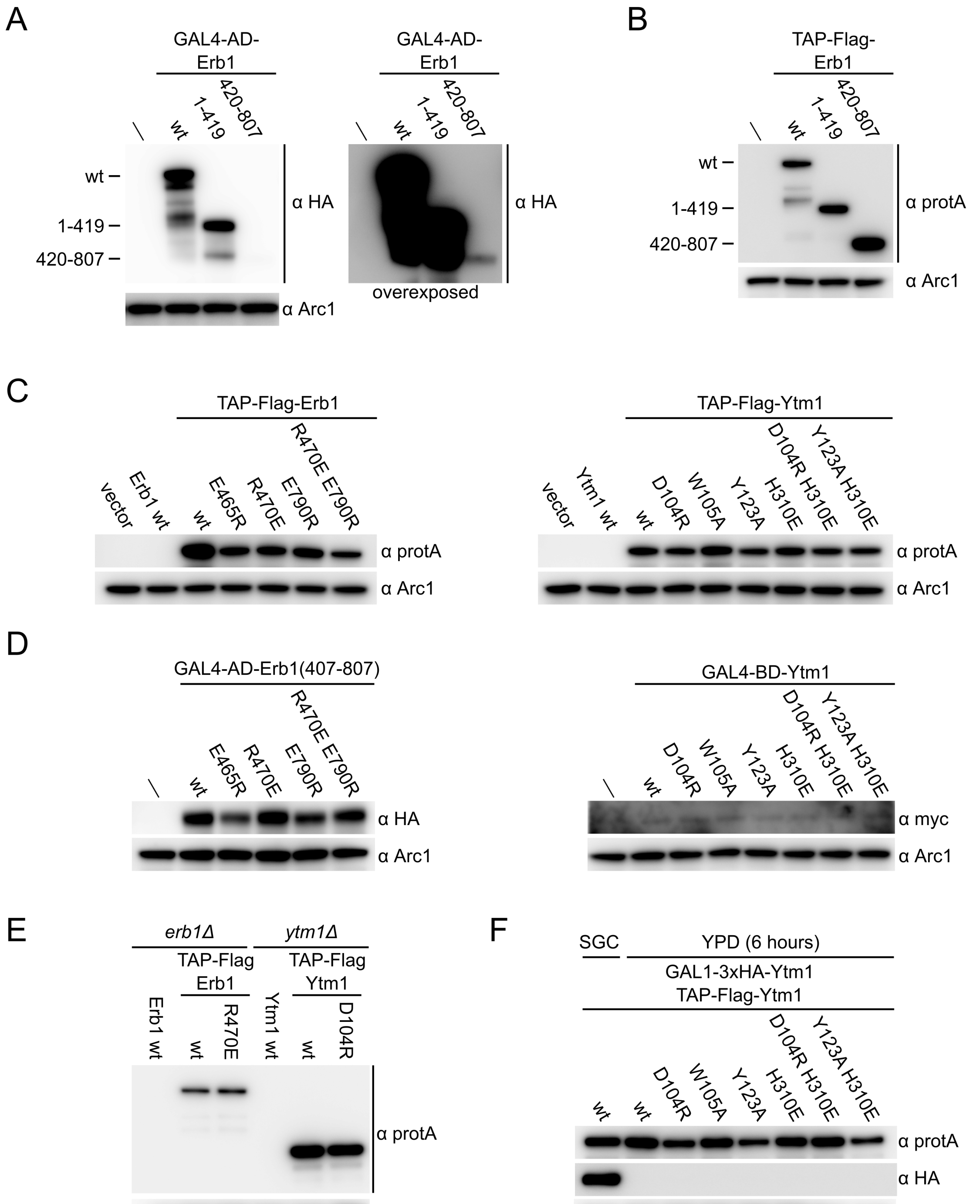
Ytm1, chain B: the first 4 residues resulting from TEV-cleavage, 0-9, 25-26, 133-136, 273-282, 351-352 and 488-495

Ytm1, chain D: the first 4 residues resulting from TEV-cleavage, 0-9, 25-26, 133-136, 273-282, 351-355 and 488-495

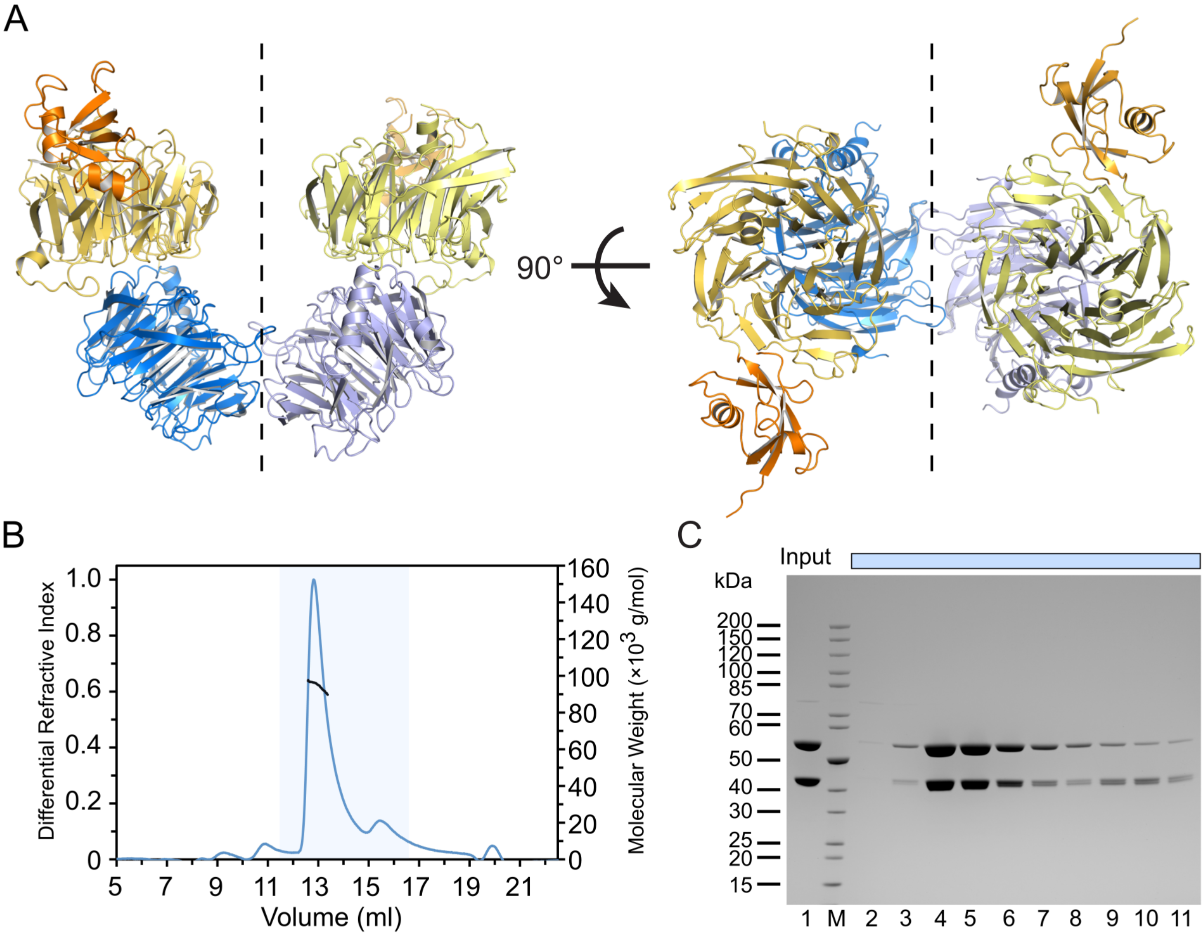


Supplementary Figure S1: The interaction of Erb1 and Ytm1 is conserved in *C. thermophilum*.

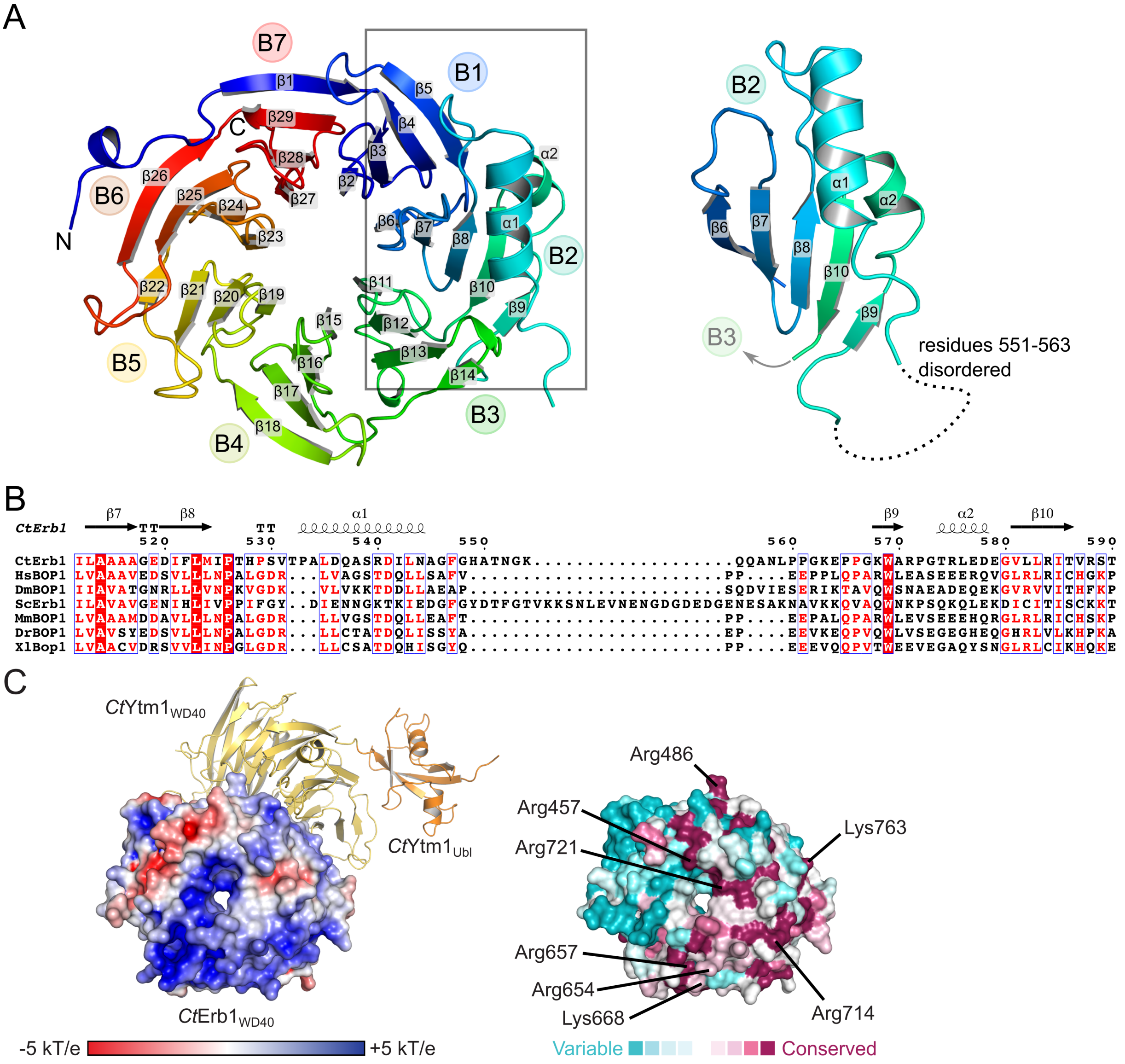
(**A**) Domain organization of Ytm1 and Erb1 from *Chaetomium thermophilum*. *Ct*Erb1 contains an N-terminal domain (grey) and a C-terminal WD40 domain (*Ct*Erb1$_{\mathrm{WD40}}$; blue). *Ct*Ytm1 contains an ubiquitin-like domain (Ubl; orange) and a β-propeller (WD40; yellow). The domain boundaries are given in residue numbers. (**B**) The C-terminal β-propeller of *Ct*Erb1 (aa423-801) interacts with *Ct*Ytm1 in Y2H. *Ct*Erb1 constructs were fused to an N-terminal GAL4-AD and *Ct*Ytm1 was fused to a N-terminal GAL4-BD. Constructs were transformed into the PJ69-4A strain. Cells were spotted in 10-fold serial dilutions and growth on SDC (SDC-Leu-Trp), SDC-His (SDC-Leu-Trp-His) and SDC-Ade (SDC-Leu-Trp-Ade) was monitored after 6 days at 30°C. (**C**) Expression levels of the used yeast 2-hybrid constructs detected by western blotting. The GAL4-AD contained an additional HA-tag and the GAL4-BD a myc-tag. Arc1 served as a loading control. (**D**) *Ct*Ytm1 binds to the *Ct*Erb1 β-propeller. *In vitro* binding assay with immobilized Flag-*Ct*Ytm1 and indicated *Ct*Erb1 wt, the N-terminal domain and the C-terminal β-propeller (Input lane 1-4). The different (His)$_6$-tagged *Ct*Erb1 constructs were expressed in *E. coli* and purified with Ni-NTA agarose. pA-TEV-Flag-*Ct*Ytm1 was overexpressed in *S. cerevisiae* and purified with IgG sepharose beads following TEV protease cleavage. The protein was immobilized on Flag agarose beads and incubated with the different Erb1 constructs in the presence of *E. coli* lysate to compete for unspecific binding. Flag beads were washed and the bound proteins were eluted with buffer supplemented with Flag peptide. Eluates (lane 5-8) were analysed by SDS-PAGE and Coomassie staining.



Supplementary Figure S2: Expression levels of constructs used in this study. Expression levels of constructs used for affinity purification, yeast 2-hybrid analysis and complementation analysis. The used antibodies are indicated. The protA antibody was used to detect TAP-Flag (ProteinA-TEVsite-CBP-Flag) tagged constructs. Yeast 2-hybrid constructs contained either a HA-tag (Gal4-AD) or myc-tag (GAL4-BD) for detection by western blotting. The Arc1 antibody was used to confirm equal loading. (**A**) related to Fig. 1B, (**B**) related to Fig. 1C, (**C**) related to Fig. 4D, (**D**) related to Fig. 4E, (**E**) related to Fig. 3 (**F**) related to Fig. 5F and G.

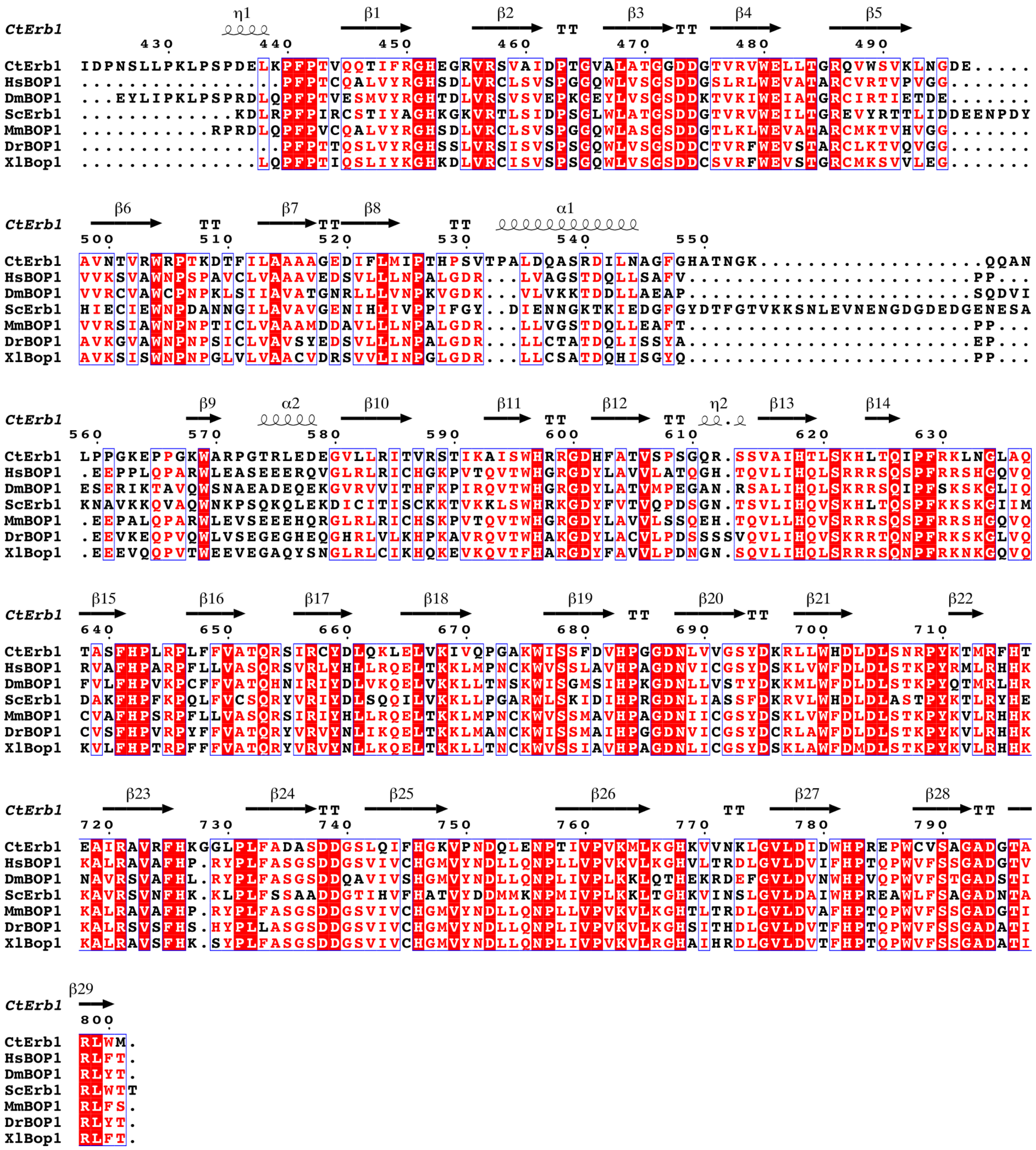


Supplementary Figure S3: Crystallographic dimer of *Ct*Erb1$_{\mathbf{WD40}}$-*Ct*Ytm1 and MALS-analysis.
(**A**) Crystal structure of the *Ct*Erb1$_{WD40}$-*Ct*Ytm1 complex with two complexes in the asymmetric unit (*Ct*Ytm1$_{WD40}$; yellow and light yellow, *Ct*Ytm1$_{Ubl}$; orange and light orange, *Ct*Erb1; marine and light blue). (**B**) Size exclusion chromatography (SEC) with online multi-angle light scattering (MALS) of the purified *Ct*Erb1$_{WD40}$-*Ct*Ytm1 complex. The blue area indicates the fractions analysed by SDS-PAGE and Coomassie staining (**C**).



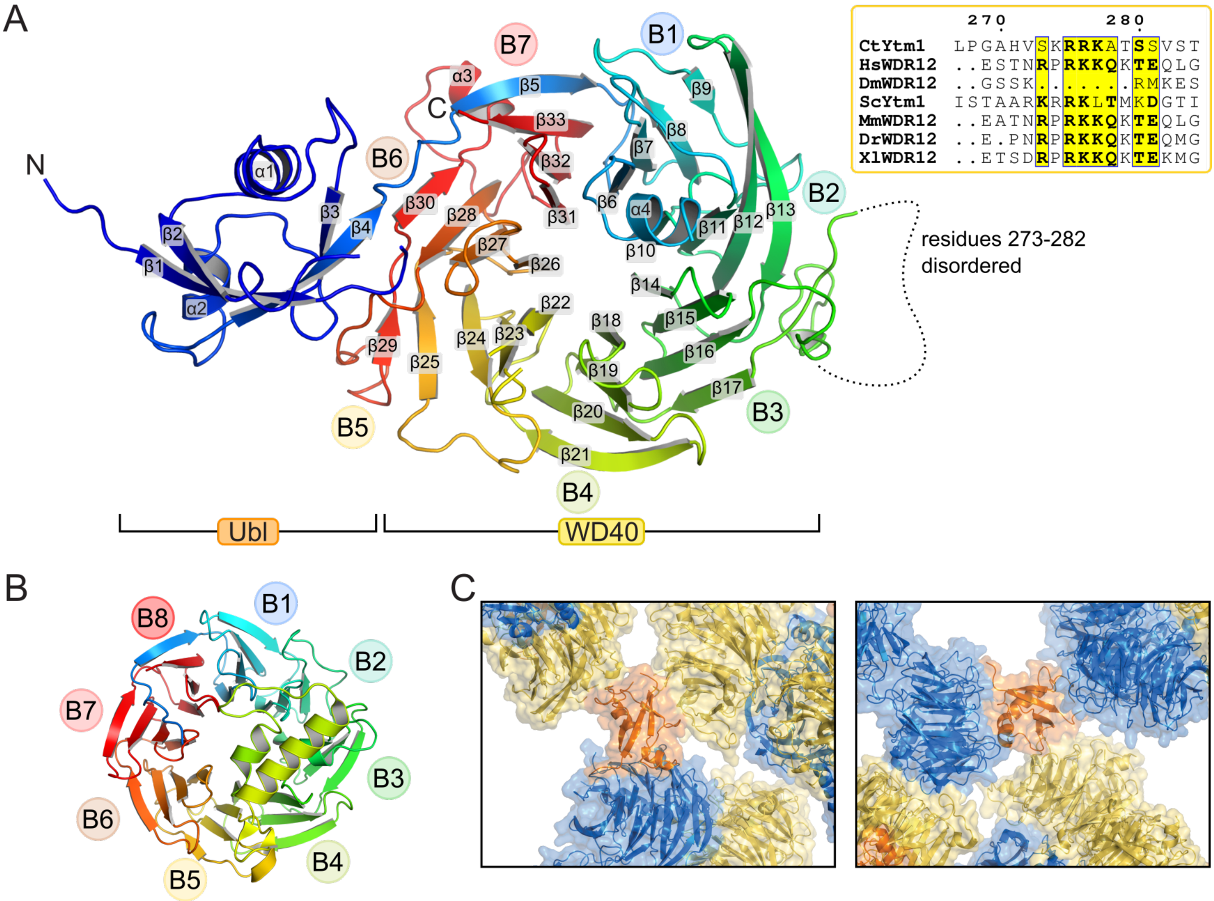
Supplementary Figure S4: Structure of *Ct*Erb1$_{\mathrm{WD40}}$ and analysis of conserved elements.

(**A**) Structure of *Ct*Erb$_{\mathrm{WD40}}$ with an insertion in blade 2 (inset), containing an additional beta-strand and two alpha helices. (**B**) Multiple sequence alignments of Erb1 blade 2 (Bop1 in higher eukaryotes). The sequences of *Chaetomium thermophilum* (Ct), *Homo sapiens* (Hs), *Drosophila melanogaster* (Dm), *Saccharomyces cerevisiae* (Sc), *Mus musculus* (Ms), *Danio rerio* (Dr) and *Xenopus laevis* (Xl) were aligned using Clustal Omega and visualized with ESPRIPT. (**C**) Positive patch on *Ct*Erb1$_{\mathrm{WD40}}$ opposite to the interaction surface to *Ct*Ytm1 (left) and ConSurf analysis (right) of *Ct*Erb1$_{\mathrm{WD40}}$, the conserved positive residues are labelled. Variable amino acids are coloured in turquoise and conserved amino acids in maroon. The colour-coding bar is shown below.

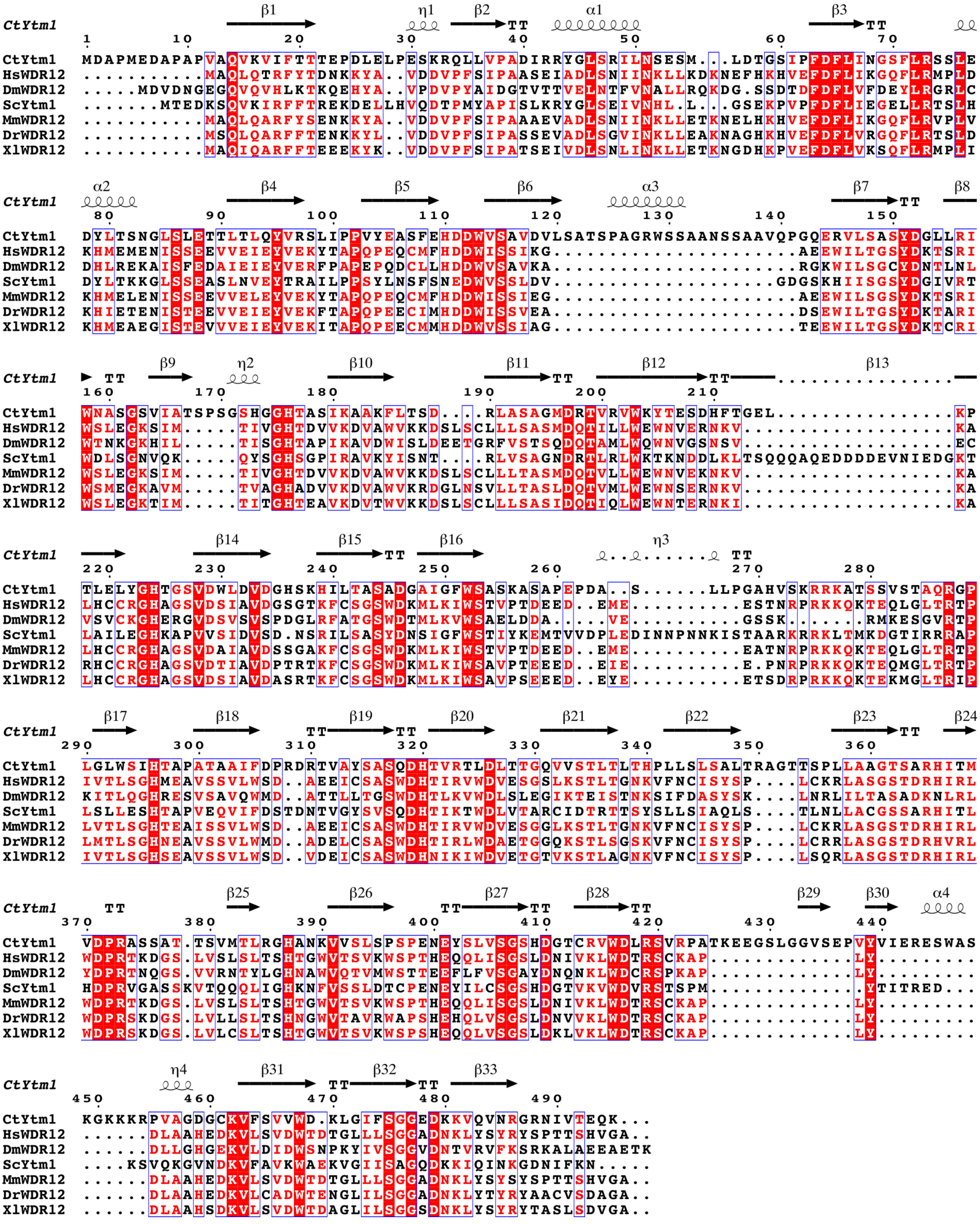


Supplementary Figure S5: Multiple sequence alignment of Erb1.

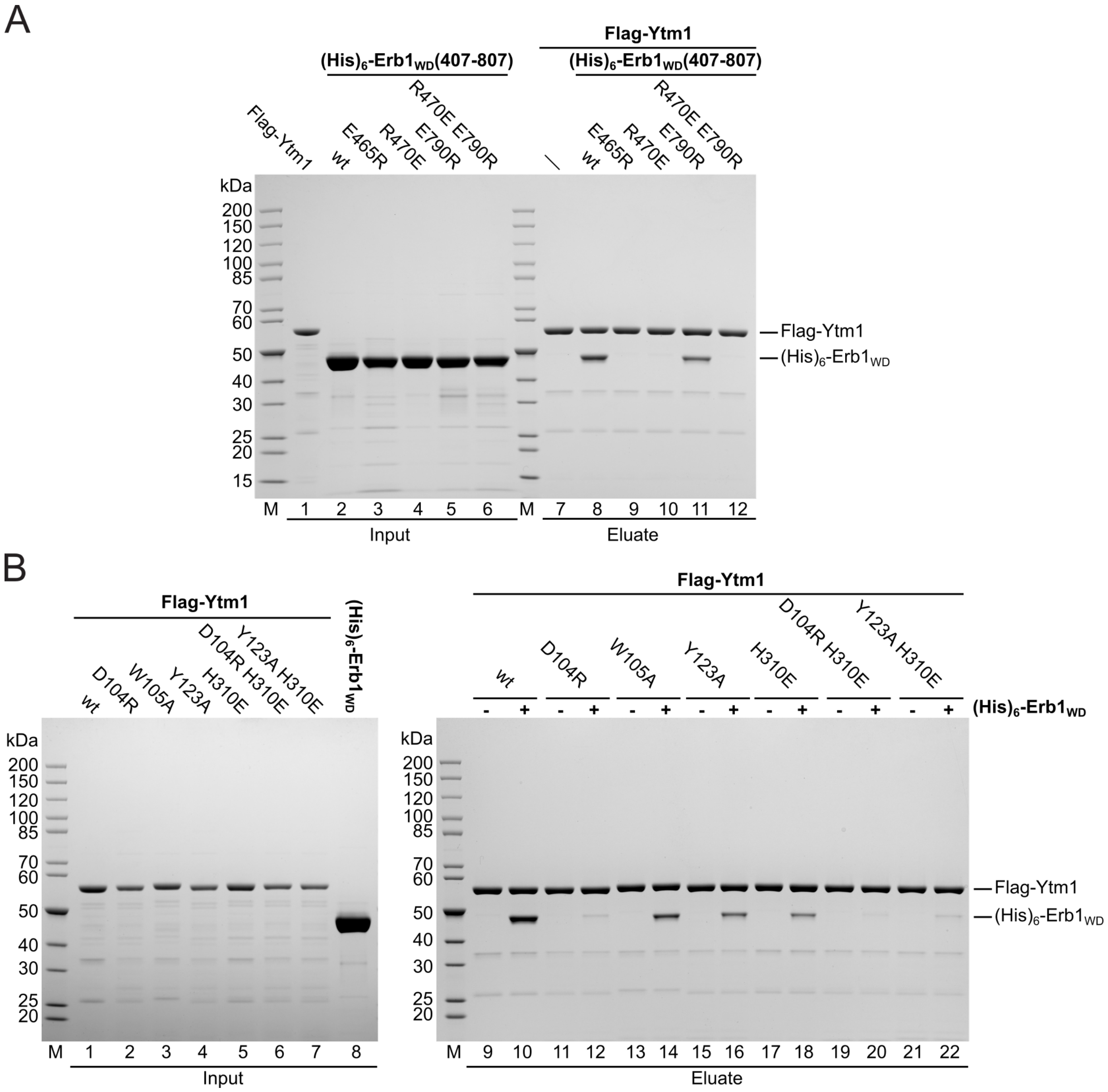
Multiple sequence alignments of Erb1 (named Bop1 in higher eukaryotes). The sequences of *C. thermophilum* (Ct), *Homo sapiens* (Hs), *Drosophila melanogaster* (Dm), *S. cerevisiae* (Sc), *Mus musculus* (Ms), *Danio rerio* (Dr) and *Xenopus laevis* (Xl) were aligned using Clustal Omega and visualized with ESPRIPT. Highly conserved residues are highlighted (red boxes).



Supplementary Figure S6: Structure of *Ct*Ytm1, comparison with *Ct*Rsa4 and crystal packing of the Ubl-domain.
(**A**) Structure of *Ct*Ytm1 with an additional beta-strand in blade 5 and two alpha helices in blade 1 and 6. The putative NLS located in blade 3 is not resolved in the structure and is indicated by a dashed line, sequence alignment of the corresponding region is given in the inset. Multiple sequence alignments of Ytm1 (WDR12 in higher eukaryotes), conserved residues belonging to the putative NLS are boxed yellow. The sequences of *Chaetomium thermophilum* (Ct), *Homo sapiens* (Hs), *Drosophila melanogaster* (Dm), *Saccharomyces cerevisiae* (Sc), *Mus musculus* (Ms), *Danio rerio* (Dr) and *Xenopus laevis* (Xl) were aligned using Clustal Omega and visualized with ESPRIPT. (**B**) Structure of *Ct*Rsa4$_{\text{WD40}}$ (PDB-ID: 4WJS), the Ubl domain has been omitted for clarity, the blades are numbered with B1-8. (**C**) The Ubl-domain of chain B is held in place by interactions with one *Ct*Erb1$_{\text{WD40}}$ and another *Ct*Ytm1 molecule (left), whereas the Ubl domain of chain D is stabilized by interactions with two other *Ct*Erb1$_{\text{WD40}}$ molecules (right).

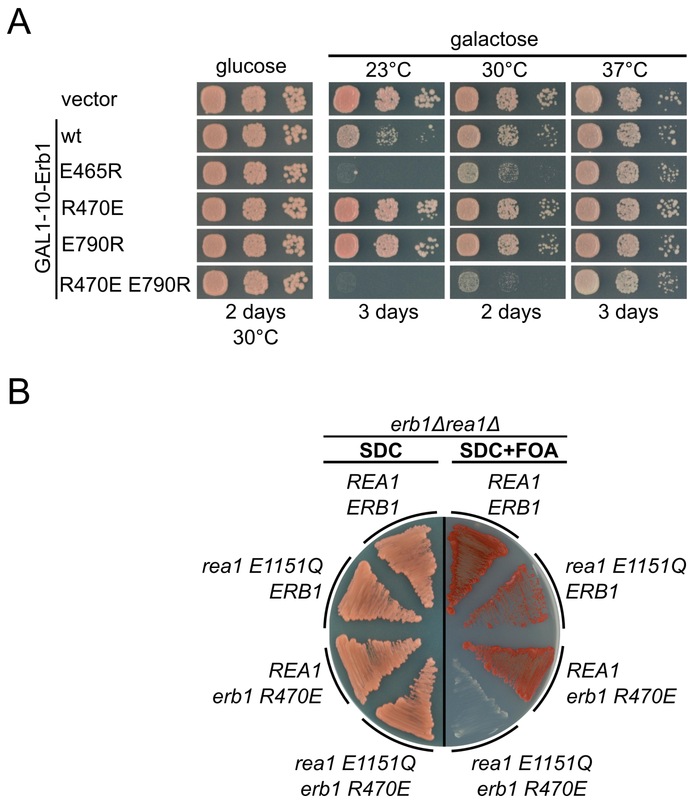


Supplementary Figure S7: Multiple sequence alignment of Ytm1.
Multiple sequence alignment of Ytm1 (named WDR12 in higher eukaryotes). The sequences of *C. thermophilum* (Ct), *Homo sapiens* (Hs), *Drosophila melanogaster* (Dm), *S. cerevisiae* (Sc), *Mus musculus* (Ms), *Danio rerio* (Dr) and *Xenopus laevis* (Xl) were aligned using Clustal Omega and visualized with ESPRIPT. Highly conserved residues are highlighted (red boxes).

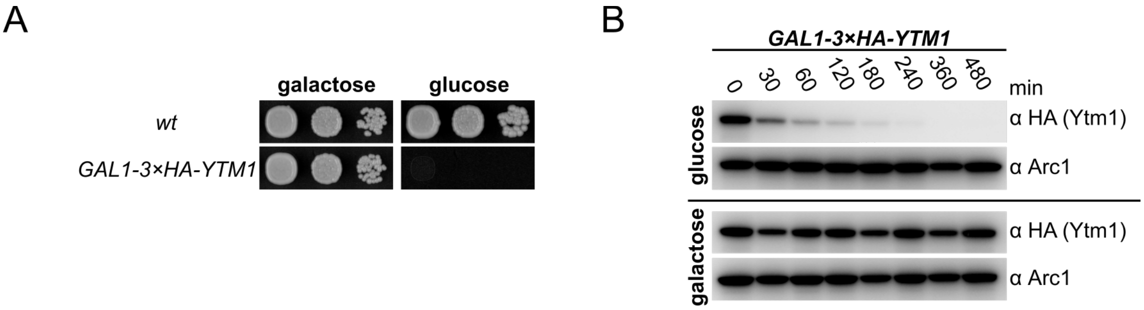


Supplementary Figure S8: Erb1/Ytm1 Interface analysed by *in vitro* binding assays.

Binding assay between Ytm1 and the Erb1$_{WD}$ (aa407-807) from *S. cerevisiae*. The different Erb1-Ytm1 interface mutations are indicated. The different Flag-Ytm1 alleles were overexpressed and purified from *S. cerevisiae*. The (His)$_6$-tagged Erb1$_{WD}$ wt construct and the different mutant constructs were expressed and purified from *E. coli*. (**A**) Binding assay with Flag-Ytm1 and the indicated Erb1$_{WD}$ alleles. Inputs (lane 1-6) and eluates (lane 7-12) are shown. (**B**) Binding assay between the different Flag-Ytm1 alleles and Erb1$_{WD}$ wt. Left: Inputs used for *in vitro* binding assay (lane 1-8). Right: Final eluates (lane 9-22).

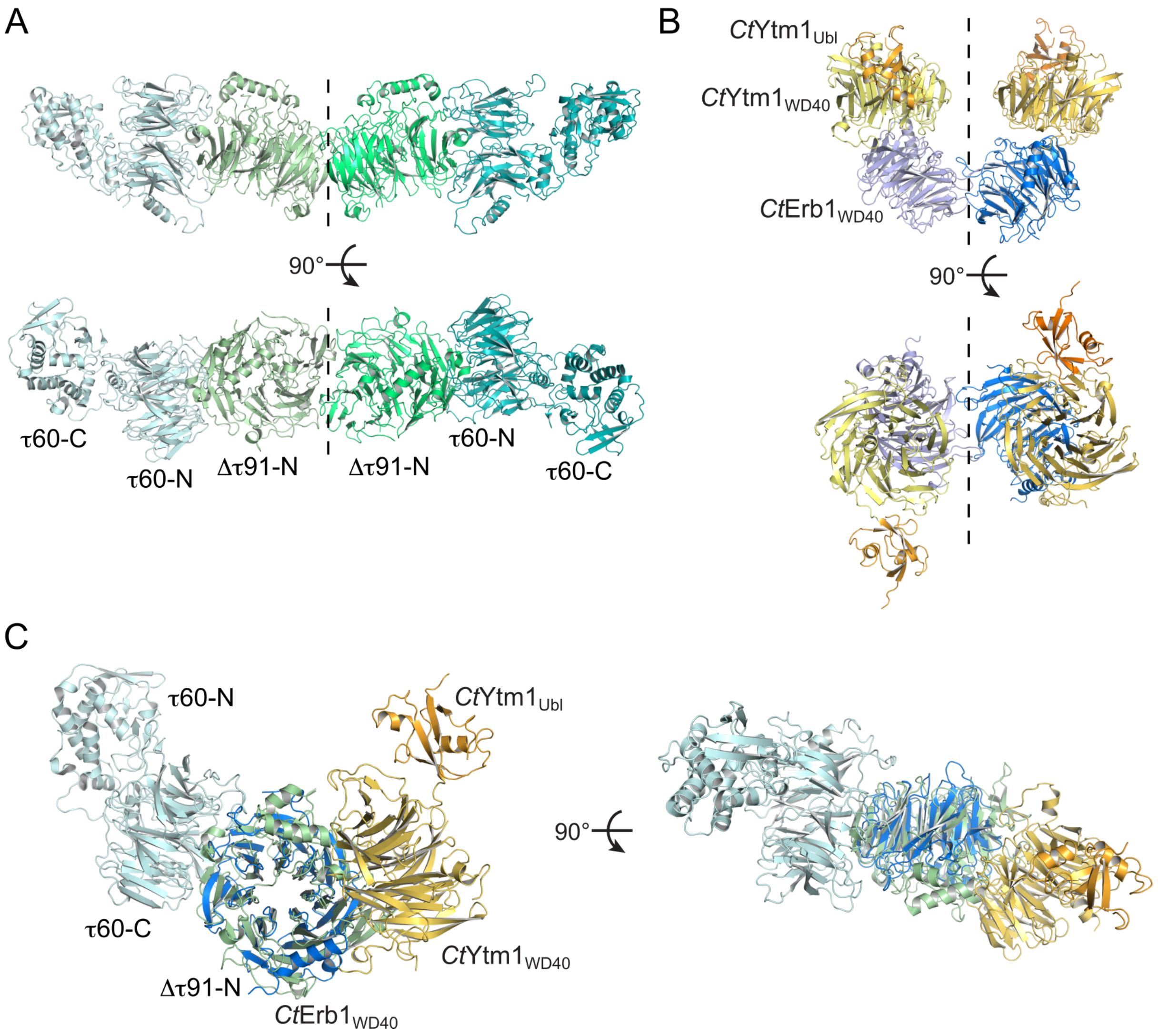


Supplementary Figure S9: Overexpression of Erb1 alleles and genetic interaction of Erb1-R470E with Rea1-E1151Q.
(**A**) Plasmids harbouring the different Erb1 alleles under control of the *GAL1-10* promoter were transformed in a yeast wild-type strain (W303). Cells were spotted in 10-fold serial dilutions on glucose (SDC-Leu) or galactose (SGC-Leu) containing medium and growth at the indicated temperatures was monitored. (**B**) Synthetic lethal interaction between *TAP-Flag-erb1 R470E* and *rea1 E1151Q*. The *erb1Δ rea1Δ* double shuffle strain was transformed with the indicated Rea1 and TAP-Flag-Erb1 alleles. Cells were streaked on SDC (SDC-Leu-Trp) and SDC+FOA plates and growth at 30°C was monitored after 3 and 5 days respectively.



Supplementary Figure S10: Depletion of *YTM1* under the control of the repressible *GAL1* promoter.

(**A**) Growth of the *GAL1-3×HA-YTM1* strain compared to wt (W303) on galactose containing medium (YPG) and on glucose containing medium (YPD). Cells were spotted in 10-fold serial dilutions and growth was analysed after 2 days at 30°C. (**B**) Western blot analysis of *GAL1-3×HA-YTM1* in glucose and galactose medium. The HA antibody was used to detect Ytm1 protein levels and the antibody against Arc1 served as a loading control.



Supplementary Figure S11: Comparison of the Erb1$_{WD40}$-Ytm1 complex with the τ60/Δτ91-complex.
(**A**) Crystal structure of the τ60/Δτ91-subcomplex of yeast transcription factor IIIC (PDB-ID: 2J04). (**B**) Crystal structure of Erb$_{WD40}$-Ytm1 complex. Both structures belong to the space group *P*2$_1$ with two complexes in the asymmetric unit, related by a near 180° rotational non-crystallographic symmetry. (**C**) Overlay of both complexes via Δτ91-N/Erb1, binding of second WD40-protein proceeds in both cases via the sides of the central WD40-domain.

Supplementary Table S1: Yeast strains used in this study.

Nr.	Name	Relevant genotype	Source
Y5303	PJ69-4A	*MATα, trp1-901, leu2-3,112, ura3-52, his3-200, gal4Δ, gal80Δ, LYS2::GAL1-HIS3, GAL2-ADE2, met2::GAL7-lacZ*	30
Y3609	W303	wild type	27
Y2197	DS1-2b	wild type	5
Y5270	*erb1Δ*	*erb1*::natNT2, pRS316-*ERB1*	This study
Y5004	*ytm1Δ*	*ytm1*::kanMX6, pRS416-*YTM1*	This study
Y4502	*ytm1Δ rea1Δ*	*ytm1*::hphNT1, *rea1*::His3MX6	24
Y5758	*erb1Δ rea1Δ*	*erb1*::natNT2, *rea1*::kanMX6	This study
Y5729	*GAL1-3xHA-YTM1*	P_{GAL1}-3×HA-*YTM1*::His3MX6	This study

Supplementary Table S2: Plasmids used in this study.

Name	Relevant information	Source
pVA3-1	2µ, *TRP1*, GAL4 BD-murine *p53* (72-390aa), pGBT9	Takara Bio Inc.
pTD1-1	2µ, *LEU2*, GAL4 AD-*SV40* large T-antigen (84-708aa), pACT2	Takara Bio Inc.
pFA6A-natNT2	for genomic deletion disruption	28
pFA6A-kanMX6	for genomic deletion disruption	29
pFA6a-His3MX6-PGAL1-3×HA	P*GAL1*, 3×HA, for genomic N-terminal tagging	29
pET-24d-(His)6-CtErb1 (1-801)	Kan^r, T7 promoter, *lac* operator, N-terminal (His)$_6$ tag	This study
pET-24d-(His)6-CtErb1$_{N}$ (1-454)	Kan^r, T7 promoter, *lac* operator, N-terminal (His)$_6$ tag	This study
pET-24d-(His)6-CtErb1$_{WD}$ (423-801)	Kan^r, T7 promoter, *lac* operator, N-terminal (His)$_6$ tag	This study
YEplac112 Leu2d-PGAL1-10-pA-TEV-CtYtm1	2µ, *TRP1*, *LEU2D*, P*GAL1-10*, T*ADH1*, N-terminal pA-TEV tag	This study
pGADT7-CtErb1 (1-801)	2µ, *LEU2*, P*ADH1*, T*ADH1*, N-terminal G4AD	This study
pGADT7-CtErb1$_{N}$ (1-454)	2µ, *LEU2*, P*ADH1*, T*ADH1*, N-terminal G4AD	This study
pGADT7- CtErb1$_{WD}$ (423-801)	2µ, *LEU2*, P*ADH1*, T*ADH1*, N-terminal G4AD	This study
pGBKT7-CtYtm1	2µ, *TRP1*, P*ADH1*, T*ADH1*, N-terminal G4BD	This study
pRS316-Erb1	CEN, *URA3*, P*ERB1*, T*ERB1*	This study
pRS416-Ytm1	CEN, *URA3*, P*YTM1*, T*YTM1*	24
YCplac111-Erb1	CEN, *LEU2*, P*ERB1*, T*ADH1*	This study
YCplac111-Erb1 (1-419)	CEN, *LEU2*, P*ERB1*, T*ADH1*	This study
YCplac111-Erb1 (420-807)	CEN, *LEU2*, P*ERB1*, T*ADH1*	This study
YCplac111-TAP-Flag-Erb1	CEN, *LEU2*, P*ERB1*, T*ADH1*, N-terminal TAP-Flag tag	This study
YCplac111-TAP-Flag-Erb1 (1-419)	CEN, *LEU2*, P*ERB1*, T*ADH1*, N-terminal TAP-Flag tag	This study
YCplac111-TAP-Flag-Erb1 (420-807)	CEN, *LEU2*, P*ERB1*, T*ADH1*, N-terminal TAP-Flag tag	This study
YCplac111-TAP-Flag-Erb1 E465R	CEN, *LEU2*, P*ERB1*, T*ADH1*, N-terminal TAP-Flag tag	This study
YCplac111-TAP-Flag-Erb1 R470E	CEN, *LEU2*, P*ERB1*, T*ADH1*, N-terminal TAP-Flag tag	This study
YCplac111-TAP-Flag-Erb1 E790R	CEN, *LEU2*, P*ERB1*, T*ADH1*, N-terminal TAP-Flag tag	This study
YCplac111-TAP-Flag-Erb1 R470E E790R	CEN, *LEU2*, P*ERB1*, T*ADH1*, N-terminal TAP-Flag tag	This study
YCplac111-Ytm1	CEN, *LEU2*, P*YTM1*, T*ADH1*	This study
YCplac111-TAP-Flag-Ytm1	CEN, *LEU2*, P*YTM1*, T*ADH1*, N-terminal TAP-Flag tag	This study
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YCplac111-TAP-Flag-Ytm1 Y123A	CEN, *LEU2*, P*YTM1*, T*ADH1*, N-terminal TAP-Flag tag	This study
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YCplac111-TAP-Flag-Ytm1 D104R H310E	CEN, *LEU2*, P*YTM1*, T*ADH1*, N-terminal TAP-Flag tag	This study
YCplac111-TAP-Flag-Ytm1 Y123A E310E	CEN, *LEU2*, P*YTM1*, T*ADH1*, N-terminal TAP-Flag tag	This study
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YCplac22-Rea1 E1151Q	CEN, *TRP1*, P*REA1*, T*REA1*,	This study
pG4ADHAN111-Erb1	CEN, *LEU2*, P*ADH1*, T*ADH1*, N-terminal G4AD	This study
pG4ADHAN111-Erb1 (1-419)	CEN, *LEU2*, P*ADH1*, T*ADH1*, N-terminal G4AD	This study
pG4ADHAN111-Erb1 (420-807)	CEN, *LEU2*, P*ADH1*, T*ADH1*, N-terminal G4AD	This study
pG4ADHAN111-Erb1$_{WD}$ (407-807)	CEN, *LEU2*, P*ADH1*, T*ADH1*, N-terminal G4AD	This study
pG4ADHAN111-Erb1$_{WD}$ (407-807) E465R	CEN, *LEU2*, P*ADH1*, T*ADH1*, N-terminal G4AD	This study
pG4ADHAN111-Erb1$_{WD}$ (407-807) R470E	CEN, *LEU2*, P*ADH1*, T*ADH1*, N-terminal G4AD	This study
pG4ADHAN111-Erb1$_{WD}$ (407-807) E790R	CEN, *LEU2*, P*ADH1*, T*ADH1*, N-terminal G4AD	This study
pG4ADHAN111-Erb1$_{WD}$ (407-807) R470E E790R	CEN, *LEU2*, P*ADH1*, T*ADH1*, N-terminal G4AD	This study
pG4BDN22-Ytm1	CEN, *TRP1*, P*ADH1*, T*ADH1*, N-terminal G4BD	This study
pG4BDN22-Ytm1 D104R	CEN, *TRP1*, P*ADH1*, T*ADH1*, N-terminal G4BD	This study
pG4BDN22-Ytm1 W105A	CEN, *TRP1*, P*ADH1*, T*ADH1*, N-terminal G4BD	This study
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pG4BDN22-Ytm1 D104R H310E	CEN, *TRP1*, P*ADH1*, T*ADH1*, N-terminal G4BD	This study
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YCplac111-PGAL1-10-Ytm1 W105A	CEN, *LEU2*, P*GAL1-10*, T*ADH1*	This study
YCplac111-PGAL1-10-Ytm1 Y123A	CEN, *LEU2*, P*GAL1-10*, T*ADH1*	This study
YCplac111-PGAL1-10-Ytm1 H310E	CEN, *LEU2*, P*GAL1-10*, T*ADH1*	This study
YCplac111-PGAL1-10-Ytm1 D104R H310E	CEN, *LEU2*, P*GAL1-10*, T*ADH1*	This study
YCplac111-PGAL1-10-Ytm1 Y123A H310E	CEN, *LEU2*, P*GAL1-10*, T*ADH1*	This study
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YCplac111-PGAL1-10-Ytm1 E80A W105A	CEN, *LEU2*, P*GAL1-10*, T*ADH1*	This study
YCplac111-PGAL1-10-Ytm1 E80A Y123A	CEN, *LEU2*, P*GAL1-10*, T*ADH1*	This study
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YCplac111-PGAL1-10-Erb1	CEN, *LEU2*, P*GAL1-10*, T*ADH1*	This study
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YCplac111-PGAL1-10-Erb1 R470E	CEN, *LEU2*, P*GAL1-10*, T*ADH1*	This study
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YCplac111-PGAL1-10-Erb1 R470E E790R	CEN, *LEU2*, P*GAL1-10*, T*ADH1*	This study
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YCplac111-eGFP-Ytm1 D104R H310E	CEN, *LEU2*, P*YTM1*, T*ADH1*, N-terminal eGFP tag	This study
YCplac111-eGFP-Ytm1 Y123A H310E	CEN, *LEU2*, P*YTM1*, T*ADH1*, N-terminal eGFP tag	This study
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YEplac112 Leu2d-PGAL1-10-pA-TEV-Flag-Ytm1 D104R H310E	2µ, *TRP1*, *LEU2D*, P*GAL1-10*, T*ADH1*, N-term pA-TEV-Flag tag	This study
YEplac112 Leu2d-PGAL1-10-pA-TEV-Flag-Ytm1 Y123A H310E	2µ, *TRP1*, *LEU2D*, P*GAL1-10*, T*ADH1*, N-term pA-TEV-Flag tag	This study
pET-24d-(His)6-Erb1$_{WD}$(407-807)	Kan^r, T7 promoter, *lac* operator, N-terminal (His)$_6$ tag	This study
pET-24d-(His)6-Erb1$_{WD}$(407-807) E465R	Kan^r, T7 promoter, *lac* operator, N-terminal (His)$_6$ tag	This study
pET-24d-(His)6-Erb1$_{WD}$(407-807) R470E	Kan^r, T7 promoter, *lac* operator, N-terminal (His)$_6$ tag	This study
pET-24d-(His)6-Erb1$_{WD}$(407-807) E790R	Kan^r, T7 promoter, *lac* operator, N-terminal (His)$_6$ tag	This study
pET-24d-(His)6-Erb1$_{WD}$(407-807) E470E E790R	Kan^r, T7 promoter, *lac* operator, N-terminal (His)$_6$ tag	This study