Probe name	5'-3' sequence
Probe b	CATGGCTTAATCTTTGAGAC
Probe c	GACTCTCCATCTCTTGTCTTCTTG
Probe d	TGTTACCTCTGGGCCC
Probe e	TTTCGCTGCGTTCTTCATC
Probe f	GGCCAGCAATTTCAAGTTA
Probe g	GAACATTGTTCGCCTAGA
Probe h	CTCCGCTTATTGATATGC
Probe 5S	GGTCACCCACTACACTACTCGG

Supplementary Table S1. Oligonucleotides used as probes for RNA analysis.

	ChErb1 ₄₃₅₋₈₀₁ /ChYtm1	ChErb1 ₄₃₂₋₈₀₁ /ChYtm1	ChErb1[R486E] ₄₃₅₋₈₀₁ /ChYtm1
Data collection			
Space group	P 6₅ 2 2	P 2 ₁ 2 ₁ 2	P 6₅ 2 2
Cell dimensions a, b, c (Å) α, β, γ (°) Resolution (Å) R_{merge} $I / \sigma I$ Completeness (%) Redundancy	169.11, 169.11, 154.04 90, 90, 120 48.82 - 3.1 (3.31 - 3.1)* 0.282 (2.838) 17 (2.5) 100.00 (100.00) 39.1 (40.2)	86.443, 108.149, 108.618 90, 90, 90 48.53 - 2.1 (2.175 - 2.1)* 0.05838 (0.924) 18.69 (2.18 99.90 (99.97) 6.5 (6.5)	170.75, 170.75, 155.66 90, 90, 120 85.38-3.0 (3.18-3.0)* 0.176 (1.862 13.5 (1.6) 100.00 (100.00) 12.2 (12.7)
Refinement			
Resolution (Å) No. reflections <i>R</i> _{work} / <i>R</i> _{free} No. atoms Protein Ligand/ion Water <i>B</i> -factors Protein Ligand/ion Water R.m.s. deviations	48.82 - 3.1 (3.31 - 3.1) 24180 (4313) 0.1936 (0.2940)/ 0.2390 (0.3557) 6170 6168 2ª 0 102.40 102.40 102.40 96.10	48.53 - 2.1 (2.175 - 2.1) 60041 (5923) 0.1639 (0.2386)/ 0.2132 (0.2703) 6631 6279 26 ^b 326 63.20 63.40 76.70 57.80	85.38-3.0 (3.18-3.0) 27367 (4333) 0.2016 (0.3153)/ 0.2621 (0.3914) 6182 6177 5° 0 89.70 89.70 84.70
Bond lengths (Å) Bond angles (°)	0.006 1.26	0.008 1.18	0.006 1.35

Supplementary table S2. Data collection and refinement statistics

*Values in parentheses are for highest-resolution shell.

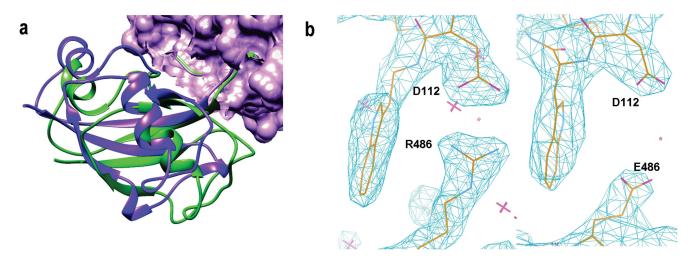
^a) Ligands: chloride ion

^b) Ligands: chloride ion, sodium ion, glycerol, ethylene glycol

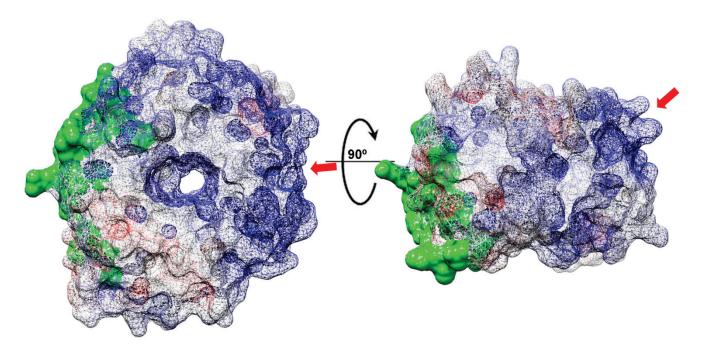
^c) Ligands: chloride ion. Data were collected from one crystal for each dataset.

	$\mathbf{x}\mathbf{x}\mathbf{x}\mathbf{x}\mathbf{x}\mathbf{x}\mathbf{x}\mathbf{x}\mathbf{x}\mathbf{x}$
Ct/1-495	1 MD AP MED AP AP V AQVKV I F TTTEPDLELPESKRQLLVP AD I RR YGLSR I LNSESMLD TGS I PF 63
Sc/1-460	1 MTE DKSQVK I RF F TREKDELLHVQD TPMYAP I SLKRYGLSE I VNHLL -GS EKPVPF 55
Sp/1-440	1 MD AQ S AP S G Q V Q V R F T T R N E D L AV G D T P I F V P T S L K R Y G L S Q I V N H L L - K T E K P T P F 56
Hs/1-423	1 M AQLQTRFYTDNKKY AVDDVPFSIP AASE I ADLSNIINKLL-KDKNEFHKHVEF53
Rn/1-423	1 M AQLQARF YTENKKY AVDDVPFSIP ATSEVADLSNIINKLL - ETKNELHKHVEF 53
Dr/1-422	1 M SQLQARFFTENKKY LVDDVPFS I PASSEVADLSGV I NKLL - EAKNAGHKHVEF 53
	$\beta_3 - \beta_4 - \alpha_4 - \beta_5 - 1d - 1a - \alpha_5 - \beta_5 - $
Ct/1-495	64 DFL I NGSFLRSSLED YL TSNGL <mark>S</mark> LE TTL TLQYVRSL I PPV YE ASFEHDDWVS AVDVLS ATSP AGRWS 130
Sc/1-460	56 DFLIEGELLRTSLHDYLTKKGLSSE ASLNVEYTRAILPPSYLNSFSNEDWVSSLDVG112
Sp/1-440	57 DFLVQGQLLKTTLDEYIVQNGLS1 <mark>E</mark> 3ILDIEYIQSTLPPAYLASFSHDDWISGIQL112
Hs/1-423	54 DFL I KGQFLRMPLDKHMEMEN I SS <mark>E</mark> EVVE I EYVEKYTAPQPEQCMFHDDWI SSI KG 109
Rn/1-423	54 DFLIKGQFLRMPLVKHMELEDISS <mark>E</mark> EVVELEYVEKYTAPQPEQCMFHDDWISSIKG109
Dr/1-422	54 DFLVRGQFLRTSLDKHIETENIS <mark>1E</mark> EVVEIEYVEKFTAPQPEECIMHDDWISSVEA109
	2a
Ct/1-495	131 S AANSS AAVQP GQERVLS AS YD GLLR IWN AS GSV I ATSP SGSHGGH TAS I K AAKF L - TS DRL A192
Sc/1-460	113 DGSKH I I SG <mark>SYD</mark> GIVR TWDL SGNVQKQ YSGHSGPIR AVKYI - SN TRLV 159 113 TSDTIL TSSYDGIARVWDKSGEIKFQ STGCGSSLKSASWHIPN QSFL 159
Sp/1-440	
Hs/1-423 Rn/1-423	110 A E EWIL TG <mark>SYD</mark> KTSRIWSLEGKSIMT IVGHTDVVKDVAWV - KKDSLSCLLL 159 110 A E EWILSG <mark>SYD</mark> KTSRIWSLEGKSIMT IVGHTDVVKDVAWV - KKDSLSCLLL 159
Dr/1-422	110
D1/1-422	THU DSEWIL IGSTUK IAK IWSMEGKAVMI VAGHADVVRDVAWV - RKDGENSVEL 159
	2c 3d 3d 3d 3d
Ct/1-495	193 SAGMDRTVRVWKYTESDHFTG-EL-KPTLELYGHTGSVDWLDVDGHSKHILTA 243
Sc/1-460	160 SAGNDRTLRLWKTKNDDLKLTSQQQAQEDDDDEVNIEDGKTLAILEGHKAPVVSIDVSD-NSRILSA225
Sp/1-440	160 TASL DQK I FHWV I EEPESMLD AEKKSSGILQTLFVGHKDIVERVRSLESSSVF I SA 215
Hs/1-423	160 SASMDQTILLWEWNVERNKV KALHCCRGHAGSVDSIAVDGSGTKFCSG207
Rn/1-423	160 TASMDQTILLWEWNIERNKV
Dr/1-422	160 TASL DQTVMLWEWNSERNKV KARHCCRGH AGSVD T I AVDP TR TKF CSG 207
	-3c $-4d$ $-4d$
Ct/1-495	244 SADGA I GFWSASKASAP EPDASLLP GAHVSKRRKATSS - VSTAQRGPLGLWSIH TAPATA 302
Sc/1-460	226 SYDNSIGFWSTIYKEMTVVDPLEDINNPNNKISTAARKRRKLTMKDGTIRRRAPLSLLESHTAPVEQ292
Sp/1-440	216 SADNTVGIWDFERSPETTLESFSSSISKKRRKNAEFTPQAGARSPLILCEGHTGPVMD274
Hs/1-423	208 SWDKMLKIWSTVPTDEEDEMEESTNRPRK-KQKTE-QLGLTRTPIVTLSGHMEAVSS262
Rn/1-423	208 SWDKMLKIWSTVPTDEEDEIQEPTNRPRK-KQKTE-QLGLTRTPLVTLSGHTEAISS262
Dr/1-422	208 SWDKMLKIWSAVPTEEEDEIEEP-NRPRK-KQKTE-QMGLTRTPLMTLSGHNEAVSS261
	4a 4b 4c 5d 5a 5b 5c
Ct/1-495	303 AIF DPRDR TV AYS AS QDH TVR TL DL T TG QV VS TL TL THPLLS LS AL TR AG T SPLL AAG TS ARH I TM 369
Sc/1-460	293 VIFDSTDNTVGYSVSQDHTIKTWDLVTARCIDTRTTSYSLLSIAQLSTLNLLACGSSARHITL355
Sp/1-440	275 IVFSDDPS-VAYSVGQDHTIKTWDLITGQNVDSKITKAPLLCVEKLTDLHLVICGSSARHIVV336
Hs/1-423	263 VLWSD AEE I CS ASWDHT I RVWDVESGSLKSTL TGNKVFNC I SYSP L CKRL ASGS TDRH I RL 323
Rn/1-423	263 VLWSD ADE VCS ASWDHTVRVWDVESGGLKSTLTGNKVFNCISYSP LCKRLASGSTDRHIRL323 262 VLWMD ADE LCS ASWDHTIRLWD AE TGGQKSTLSGSKVFNCISYSP LCRRLASGSTDRHVRL322
Dr/1-422	
Ct/1-495	370 VDPRASSATTS-VMTLRGHANKVVSLSPSPENEYSLVSGSHDGTCRVWDLRSVRPATKEEGSLGGVS435
Sc/1-460	356 HDPRVGASSKVTQQQLIGHKNFVSSLDTCPENEYILCSGSHDGTVKVWDVRST408
Sp/1-440	337 HDPR AGSDK I V - SHTL SGHKNL V SGL S AS PENP YMF AS VSHDNTCRVWDVR ATS 389
Hs/1-423	324 WDPR TKDGSLV-SLSL TSH TGWV TSV KWSP THEQQL I SGSLDN I VKLWD TRSCK
Rn/1-423	324 WDPR TKDGSLV-SLSL TSH TGWV TSV KWSP THDQQL I SGSLDNMVKLWD TRSCK
Dr/1-422	323 WDPRSKDGSLV-LLSLTSHNGWVTAVRWAPSHEHQLVSGSLDNVVKLWDTRSCK
	$- \boxed{7} \boxed{\alpha} \boxed{\alpha} \boxed{2} \boxed{\alpha} \boxed{10} \boxed{7} \boxed{7} \boxed{7} \boxed{7} \boxed{7} \boxed{7} \boxed{7} 7$
Ct/1-495	436 EPV YV I ERESWASKGKKKRPV AGDGCKVF SVVWDKL - G I F SGGEDKKVQVNRGRN I V T - EQK 495
Sc/1-460	409 SPMYTITREDKSVQKGVNDKVFAVKWAEKVGIISAGQDKKIQINKGDNIFKN 460
Sp/1-440	390 GSIYTISRAEKTGSQWDKLFAVDWNKSIGIVTGGTDKQLQINQSSSF-G-KSE 440
Hs/1-423	377 APL YDL AAHEDKVL SVDWTDTGLLLSGGADNKL YS YR YSP TTSHVGA 423
Rn/1-423	377 APL YDL AAHEDKVL SVDWTDTGLLLSGGADNKL YS YR YSP TTSHVGA 423
Dr/1-422	376 APLYDLAAHEDKVLCADWTENGLILSGGADNKLYTYRYAACVSDAGA 422

Supplementary Figure S1. Multiple sequence aligment of Ytm1 orthologues. Sequences corresponding to Ytm1 (or WDR12) proteins from *Chaetomium thermophilum* (Ct), *Saccharomyces cerevisiae* (Sc), *Schizosaccharomyces pombe* (Sp), *Homo sapiens* (Hs), *Rattus norvegicus* (Rn) and *Danio rerio* (Dr) were aligned using JalView. Secondary structure elements as seen in the final model of ChYtm1 are represented above the alignment. β -strands are marked with arrows, α -helices are depicted with coils. Secondary structure elements corresponding to UBL are shown in purple. Coloring and numbering of the following β -strands correspond to the WD repeats. Conserved residues are marked with shadows. The conserved glutamic acid proposed to bind the MIDAS domain of Rea1 is marked with a green box. The sequence corresponding to the knob-like structure is shown with orange line



Supplementary Figure S2. (a) UBL of ChYtm1 presents certain degree of flexibility because it appears in two different orientations depending on the symmetry of the crystals. UBL in purple: P 21 21 2 and in green: P 65 2 2 space groups. **(b)** Upon successful crystallization of ChYtm1/ChErb1[R486E]₄₃₂₋₈₀₁ we proved that the β -propeller structure was intact and the only difference between both dimers was the lack of D112-R486 salt bridge (left) that is not formed in the mutant (right).



Supplementary Figure S3. Electrostatic surface potential of the β -propeller of Erb1 represented as mesh shows that highly electropositive area possibly involved in binding to RNA (red arrow) is located on the opposite side to Ytm1 binding site (in green)