Crystal structures of Rea1-MIDAS bound to its ribosome assembly factor ligands resembling integrin–ligand-type complexes

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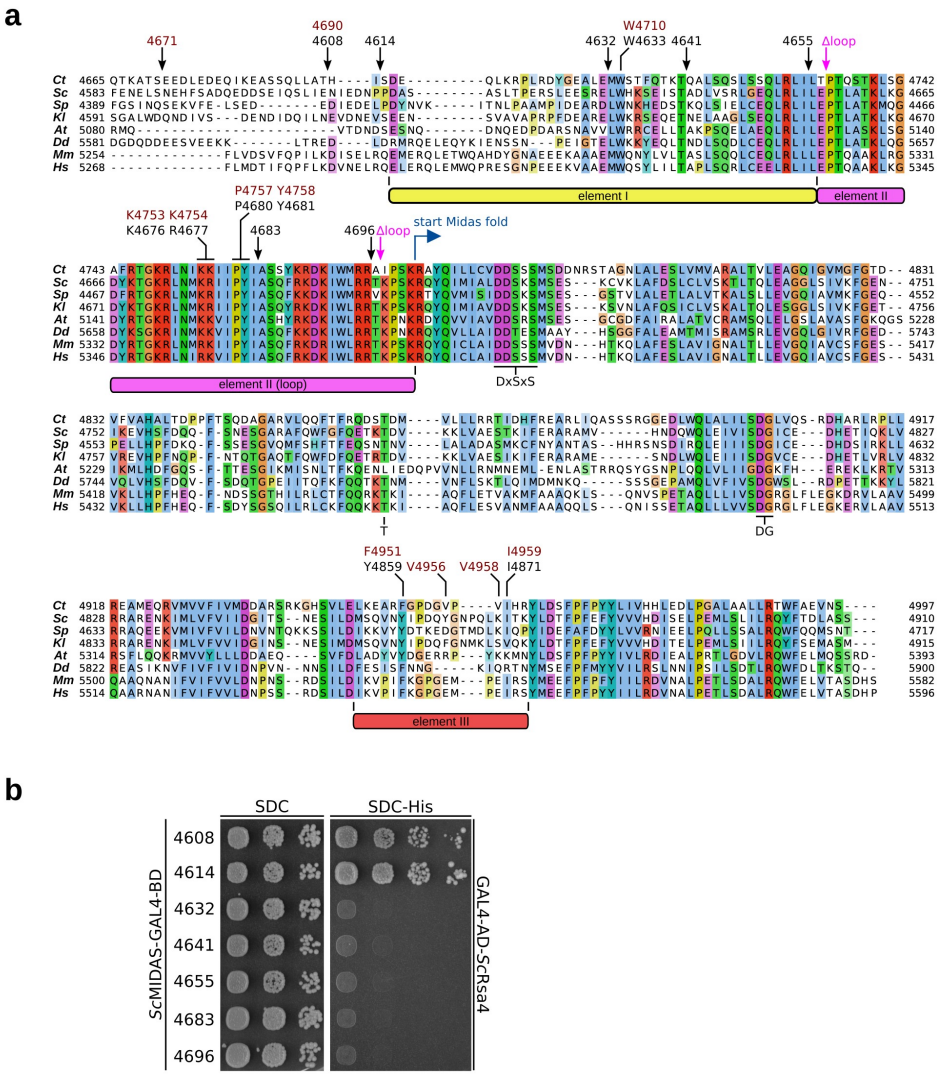
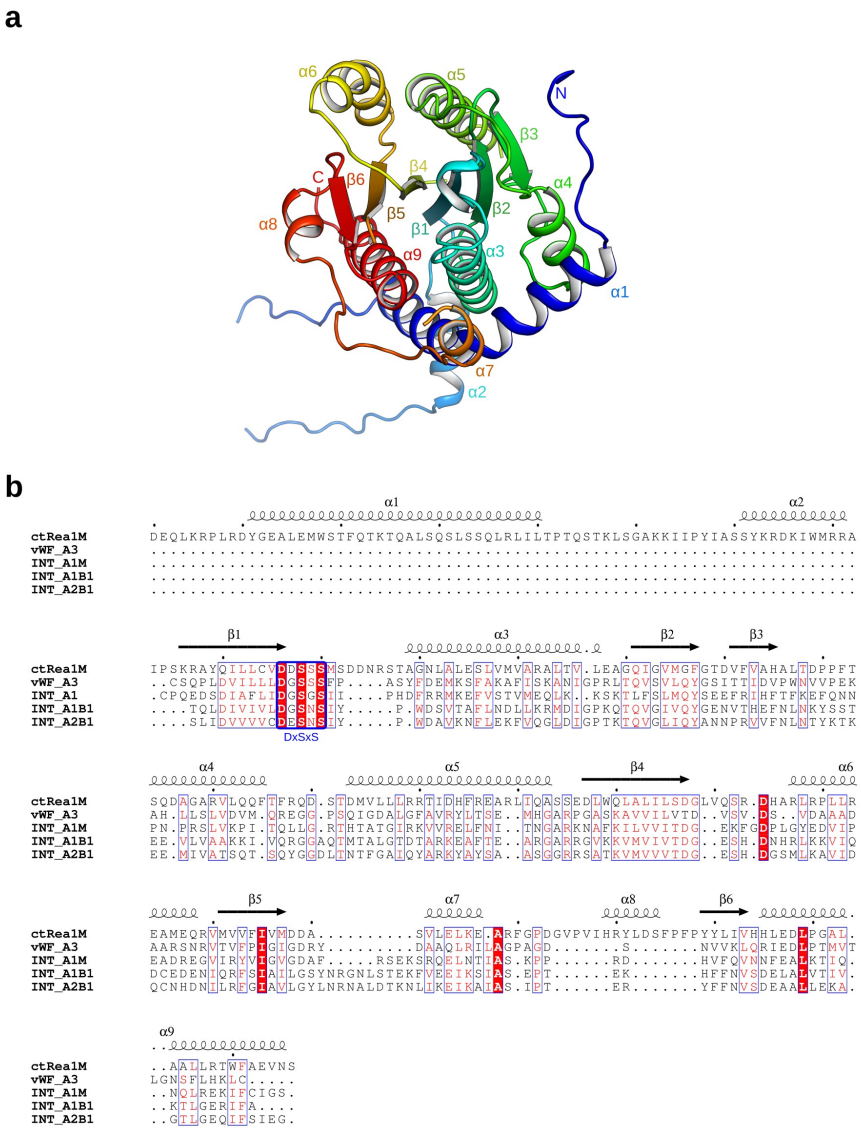
Supplementary Information

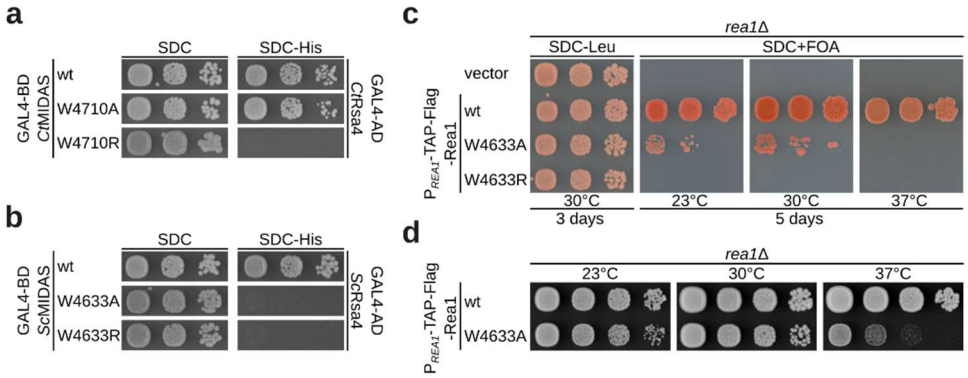
Supplementary Figures



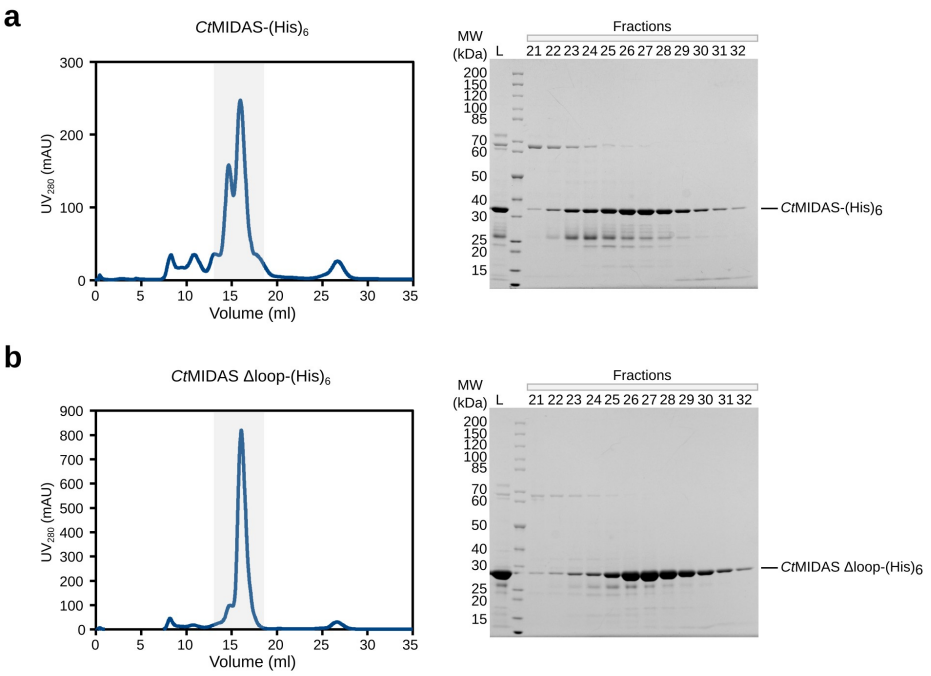
Supplementary Figure 1 Multiple sequence alignment of the Rea1-MIDAS domain and truncation analysis. (**a**) Multiple sequence alignment of Rea1 with homologs showing the MIDAS domain. The sequences of *Chaetomium thermophilum* (*Ct*), *Saccharomyces cerevisiae* (*Sc*), *Schizosaccharomyces pombe* (*Sp*), *Kluyveromyces lactis* (*Kl*), *Arabidopsis thaliana* (*At*), *Dictyostelium discoideum* (*Dd*), *Mus musculus* (*Mm*), and *Homo sapiens* (*Hs*) were aligned with Clustal Omega and visualized with Jalview. The Rea1-specific elements (I–III) and the residues required for coordination of the Mg^{2+} ion are highlighted below the alignment. Indicated above the alignment are the generated truncations (arrows) and point mutations in *C.thermophilum* (red) and *S.cerevisiae* (black). The boundaries of the ∆loop constructs are indicated in pink. (**b**) Yeast two-hybrid analysis of the interactions between the indicated MIDAS constructs with full-length Rsa4 from *S. cerevisiae*. The Rsa4 construct was fused to an N-terminal GAL4-AD (activation domain) and the MIDAS constructs were fused to a C-terminal GAL4-BD (binding domain). The residue numbers indicate the N-terminal boundary of the MIDAS constructs. Plasmids were co-transformed into yeast (PJ69-4A) and transformants were spotted in tenfold serial dilutions on SDC (SDC−Leu−Trp) and SDC−His (SDC−Leu−Trp−His) selective plates. Cell growth was monitored after incubation for 3 days at 30 °C.



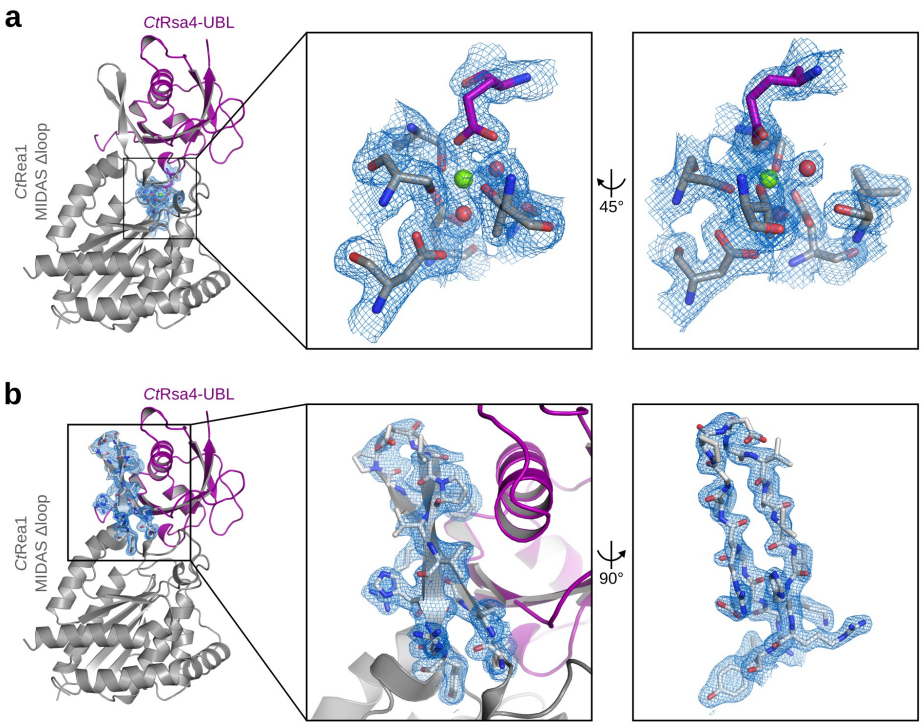
Supplementary Figure 2. Secondary structure comparison of the Rea1-MIDAS and integrin structures. (**a**) Structure of the Rea1-MIDAS domain from *C. thermophilum*. (**b**) Sequence and structure alignment between *Ct*Rea1 and depicted integrin structures. The alignment was created with MAFFTASH. The following structures were aligned: ctRea1M (*C. thermophilum* Rea1-MIDAS, sequence contains gaps not covered in the structure), vWF_A3 (von Willebrand factor A3 domain, PDB ID: 1AO3), INT_A1M (integrin alpha M I domain, PDB ID: 1MF7), INT_A1B1 (integrin alpha1beta1 I-domain, PDB ID: 1QCY) and INT_A2B1 (integrin alpha2beta1 I-domain, PDB ID: 5HJ2).



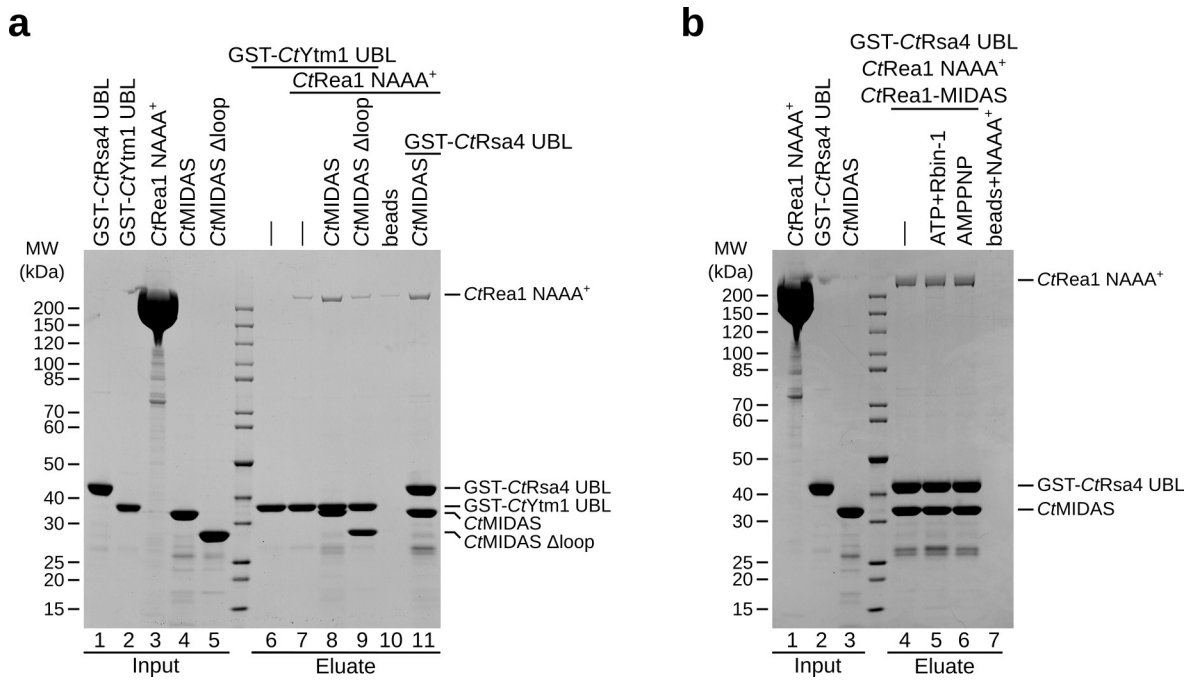
Supplementary Figure 3. Functional analysis of the conserved Try residue in the N-terminal extension of Rea1-MIDAS. (**a**,**b**) Yeast two-hybrid analysis of the Rea1-MIDAS and Rsa4 constructs from *C. thermophilum* (**a**) and *S. cerevisiae* (**b**). The MIDAS constructs were fused to an N-terminal GAL4-BD and the Rsa4 constructs to an N-terminal GAL4-AD. Transformants were spotted on SDC (SDC−Leu−Trp) plates or SDC−His (SDC−Leu−Trp−His) selective plates and cell growth was monitored after incubation for 3 days at 30 °C. (**c**) An empty plasmid control or the indicated Rea1 constructs, fused to an N-terminal TAP-Flag tag, were transformed into a *rea1*Δ shuffle strain. Cells were spotted in 10-fold serial dilutions on SDC−Leu and SDC+FOA plates and cells were grown at the indicated temperatures for 3 and 5 days, respectively. (**d**) Growth analysis of the indicated Rea1 constructs after shuffling on SDC+FOA plates. Cells were spotted in tenfold serial dilutions on YPD plates and growth at the indicated temperatures was monitored after 2 days.



Supplementary Figure 4. Purification of *Ct*MIDAS and *Ct*MIDAS Δloop. (**a**,**b**) The left-hand panels show the size-exclusion chromatography profiles of *Ct*MIDAS-$(His)_6$ (amino acids 4690-4997) (**a**) and *Ct*MIDAS Δloop-$(His)_6$ (amino acids 4690-4733-GSG-4774-4997) (**b**). The absorbance at 280 nm (mAU) was plotted against the elution volume (ml). Fractions highlighted in gray were analyzed by SDS-PAGE and Coomassie staining (right-hand panels).



Supplementary Figure 5. Structure of the *Ct*Rea1-MIDASΔloop–*Ct*Rsa4-UBL complex. (**a**,**b**) The *Ct*Rea1-MIDASΔloop–*Ct*Rsa4-UBL model and corresponding electron densities of the Mg^{2+} coordination site (**a**) and the β-hairpin of the Rea1-specific element III (**b**) contoured at 1.5 and 1.0 sigma, respectively. The *Ct*Rsa4-UBL is highlighted in purple and the *Ct*Rea1-MIDASΔloop is shown in gray. Close-up views are shown in two orientations.



Supplementary Figure 6. The MIDAS element II loop is required for interaction of the Rea1-MIDAS–Ytm1-UBL complex with the Rea1-AAA$^+$ ring. (**a**) GST-tagged *Ct*Ytm1-UBL or *Ct*Rsa4-UBL were incubated with *Ct*Rea1-MIDAS or *Ct*Rea1-MIDAS∆loop and the *Ct*Rea1-NAAA$^+$ ring domain. After washing, the GST-tagged *Ct*Ytm1- and *Ct*Rsa4-UBL domains and bound material were eluted with GSH and analyzed with SDS-PAGE and Coomassie staining. Protein inputs are shown in lanes 1–5, final eluates in lanes 6–11. (**b**) Binding assay between GST-tagged *Ct*Rsa4-UBL, *Ct*Rea1-MIDAS and *Ct*-NAAA$^+$ ring domain in the absence of nucleotides, in presence of ATP (2 mM final concentration) and the inhibitor Rbin-1 (0.1 mM final concentration), or in presence of non-hydrolysable AMPPNP (2 mM final concentration). GST-*Ct*Rsa4-UBL and bound material was eluted with GSH and analyzed with SDS-PAGE and Coomassie staining. Protein inputs are shown in lanes 1–3, final eluates in lanes 4–7.

Supplementary Table 1. *E. coli* expression plasmids used in this study

Name	Relevant information	Source
pET24d-*Ct*MIDAS-(His)$_6$	Kan^R, T7 promoter, *lac* operator, (aa 4671–4997)	This study
pETHis-(His)$_6$-*Ct*Ytm1-UBL	Kan^R, T7 promoter, *lac* operator, (aa 8–98)	This study
pETHis-(His)$_6$-*Ct*Rsa4-UBL	Kan^R, T7 promoter, *lac* operator, (aa 31–128)	This study
pET24d-(His)$_6$-GST-*Ct*Rsa4-UBL	Kan^R, T7 promoter, *lac* operator, (aa 1–128)	This study
pET24d-(His)$_6$-GST-*Ct*Ytm1-UBL	Kan^R, T7 promoter, *lac* operator, (aa 10–98)	This study
pET-15b-*Ct*MIDAS-(His)$_6$	Amp^r, T7 promoter, *lac* operator, (aa 4690–4997)	This study
pET-15b-*Ct*MIDASΔloop-(His)$_6$	Amp^r, T7 promoter, *lac* operator, (aa 4690–4733-GSG-4774–4997)	This study
pET24d-GST-*Ct*Kap104	Kan^R, T7 promoter, *lac* operator	[1]
pET-15b-*Ct*Syo1-(His)$_6$	Amp^r, T7 promoter, *lac* operator	[1]
pET-15b-*Ct*MIDAS PY>A-(His)$_6$	Amp^r, T7 promoter, *lac* operator, (aa 4690–4997)	This study
pET-15b-*Ct*MIDAS KK>A-(His)$_6$	Amp^r, T7 promoter, *lac* operator, (aa 4690–4997)	This study
pET-15b-*Ct*MIDAS PY/KK>A-(His)$_6$	Amp^r, T7 promoter, *lac* operator, (aa 4690–4997)	This study

Supplementary Table 2. *S. cerevisiae* plasmids used in this study

Name	Relevant information	Source
pVA3-1 (p53)	2μ, *TRP1*, GAL4 BD-murine p53 (aa 72–390), pGBT9	Takara Bio Inc.
pTD1-1 (SV40)	2μ, *LEU2*, GAL4 AD-SV40 large T-antigen (aa 84–708), pACT2	Takara Bio Inc.
YCplac111	CEN, *LEU2*	[2]
pGADT7-*Sc*RSA4	2μ, *LEU2*, P*ADH1*, T*ADH1*, N-terminal GAL4-AD	[3]
pG4BDC22-Gly-aa4608-*Sc*MIDAS	CEN, *TRP1*, P*ADH1*, T*ADH1*, C-terminal GAL4-BD	This study
pG4BDC22-aa4614-*Sc*MIDAS	CEN, *TRP1*, P*ADH1*, T*ADH1*, C-terminal GAL4-BD	This study
pG4BDC22-Gly-aa4632-*Sc*MIDAS	CEN, *TRP1*, P*ADH1*, T*ADH1*, C-terminal GAL4-BD	This study
pG4BDC22-aa4641-*Sc*MIDAS	CEN, *TRP1*, P*ADH1*, T*ADH1*, C-terminal GAL4-BD	This study
pG4BDC22-Gly-aa4655-*Sc*MIDAS	CEN, *TRP1*, P*ADH1*, T*ADH1*, C-terminal GAL4-BD	This study
pG4BDC22-aa4683-*Sc*MIDAS	CEN, *TRP1*, P*ADH1*, T*ADH1*, C-terminal GAL4-BD	This study
pG4BDC22-aa4696-*Sc*MIDAS	CEN, *TRP1*, P*ADH1*, T*ADH1*, C-terminal GAL4-BD	This study
pGADT7-*Ct*RSA4	2μ, *LEU2*, P*ADH1*, T*ADH1*, N-terminal GAL4-AD	[4]
pG4BDN22-aa4690-*Ct*MIDAS	CEN, *TRP1*, P*ADH1*, T*ADH1*, N-terminal GAL4-BD	This study
pG4BDN22-aa4690-*Ct*MIDAS Δloop	CEN, *TRP1*, P*ADH1*, T*ADH1*, N-terminal GAL4-BD	This study
pG4BDN22-aa4690-*Ct*MIDAS DAA	CEN, *TRP1*, P*ADH1*, T*ADH1*, N-terminal GAL4-BD	This study
pG4BDN22-aa4690-*Ct*MIDAS Δloop DAA	CEN, *TRP1*, P*ADH1*, T*ADH1*, N-terminal GAL4-BD	This study
pG4BDN22-aa4608-*Sc*MIDAS	CEN, *TRP1*, P*ADH1*, T*ADH1*, N-terminal GAL4-BD	This study
pG4BDN22-aa4608-*Sc*MIDAS Δloop	CEN, *TRP1*, P*ADH1*, T*ADH1*, N-terminal GAL4-BD	This study
pG4BDN22-aa4608-*Sc*MIDAS DAA	CEN, *TRP1*, P*ADH1*, T*ADH1*, N-terminal GAL4-BD	This study
pG4BDN22-aa4608-*Sc*MIDAS Δloop DAA	CEN, *TRP1*, P*ADH1*, T*ADH1*, N-terminal GAL4-BD	This study
pG4BDN22-aa4690-*Ct*MIDAS F4951R	CEN, *TRP1*, P*ADH1*, T*ADH1*, N-terminal GAL4-BD	This study
pG4BDN22-aa4690-*Ct*MIDAS F4951A	CEN, *TRP1*, P*ADH1*, T*ADH1*, N-terminal GAL4-BD	This study
pG4BDN22-aa4690-*Ct*MIDAS I4959R	CEN, *TRP1*, P*ADH1*, T*ADH1*, N-terminal GAL4-BD	This study
pG4BDN22-aa4690-*Ct*MIDAS I4959A	CEN, *TRP1*, P*ADH1*, T*ADH1*, N-terminal GAL4-BD	This study
pG4BDN22-aa4690-*Ct*MIDAS V4956R	CEN, *TRP1*, P*ADH1*, T*ADH1*, N-terminal GAL4-BD	This study
pG4BDN22-aa4690-*Ct*MIDAS V4956A	CEN, *TRP1*, P*ADH1*, T*ADH1*, N-terminal GAL4-BD	This study
pG4BDN22-aa4690-*Ct*MIDAS V4958R	CEN, *TRP1*, P*ADH1*, T*ADH1*, N-terminal GAL4-BD	This study
pG4BDN22-aa4690-*Ct*MIDAS V4958A	CEN, *TRP1*, P*ADH1*, T*ADH1*, N-terminal GAL4-BD	This study
pG4BDC22-Gly-aa4608-*Sc*MIDAS Y4859R	CEN, *TRP1*, P*ADH1*, T*ADH1*, N-terminal GAL4-BD	This study
pG4BDC22-Gly-aa4608-*Sc*MIDAS Y4859A	CEN, *TRP1*, P*ADH1*, T*ADH1*, N-terminal GAL4-BD	This study
pG4BDC22-Gly-aa4608-*Sc*MIDAS I4871R	CEN, *TRP1*, P*ADH1*, T*ADH1*, N-terminal GAL4-BD	This study
pG4BDC22-Gly-aa4608-*Sc*MIDAS I4871A	CEN, *TRP1*, P*ADH1*, T*ADH1*, N-terminal GAL4-BD	This study
pG4BDC22-Gly-aa4608-*Sc*MIDAS Y4859R I4871R	CEN, *TRP1*, P*ADH1*, T*ADH1*, N-terminal GAL4-BD	This study
pG4BDC22-Gly-aa4608-*Sc*MIDAS Y4859A I4871A	CEN, *TRP1*, P*ADH1*, T*ADH1*, N-terminal GAL4-BD	This study
YCplac111-P*REA1*-TAP-Flag-*REA1*	CEN, *LEU2*, P*REA1*, T*REA1*, N-terminal TAP-Flag tag	[5]
YCplac111-P*REA1*-TAP-Flag-*rea1* Y4859A	CEN, *LEU2*, P*REA1*, T*REA1*, N-terminal TAP-Flag tag	This study
YCplac111-P*REA1*-TAP-Flag-*rea1* Y4859R	CEN, *LEU2*, P*REA1*, T*REA1*, N-terminal TAP-Flag tag	This study
YCplac111-P*REA1*-TAP-Flag-*rea1* I4871A	CEN, *LEU2*, P*REA1*, T*REA1*, N-terminal TAP-Flag tag	This study
YCplac111-P*REA1*-TAP-Flag-*rea1* I4871R	CEN, *LEU2*, P*REA1*, T*REA1*, N-terminal TAP-Flag tag	This study
YCplac111-P*REA1*-TAP-Flag-*rea1* Y4859A I4871A	CEN, *LEU2*, P*REA1*, T*REA1*, N-terminal TAP-Flag tag	This study
YCplac111-P*REA1*-TAP-Flag-*rea1* Y4859R I4871R	CEN, *LEU2*, P*REA1*, T*REA1*, N-terminal TAP-Flag tag	This study
pRS314-*RSA4*	CEN, *TRP1*, P*RSA4*, T*RSA4*,	[3]
pRS314-*rsa4*-1	CEN, *TRP1*, P*RSA4*, T*RSA4*,	[3]
pRS314-*YTM1*	CEN, *TRP1*, P*YTM1*, T*YTM1*,	[6]
pRS314-*ytm1* S78L	CEN, *TRP1*, P*YTM1*, T*YTM1*,	[6]
YCplac111-P*REA1*-TAP-Flag-*rea1* ΔMIDAS	CEN, *LEU2*, P*REA1*, T*REA1*, N-terminal TAP-Flag tag (aa 1–4621)	This study
YCplac111-P*REA1*-TAP-Flag-*rea1* Δloop	CEN, *LEU2*, P*REA1*, T*REA1*, N-terminal TAP-Flag tag	This study

Name	Relevant information	Source
YCplac111-P*REA1*-TAP-Flag-*rea1* DAA	CEN, *LEU2*, P*REA1*, T*REA1*, N-terminal TAP-Flag tag	[5]
YCplac111-P*GAL1-10*-TAP-Flag-*REA1*	CEN, *LEU2*, P*REA1*, T*REA1*, N-terminal TAP-Flag tag	[5]
YCplac111-P*GAL1-10*-TAP-Flag-*rea1* ΔMIDAS	CEN, *LEU2*, P*GAL1-10*, T*REA1*, N-terminal TAP-Flag tag	This study
YCplac111-P*GAL1-10*-TAP-Flag-*rea1* Δloop	CEN, *LEU2*, P*GAL1-10*, T*REA1*, N-terminal TAP-Flag tag	This study
YCplac111-P*GAL1-10*-TAP-Flag-*rea1* DAA	CEN, *LEU2*, P*GAL1-10*, T*REA1*, N-terminal TAP-Flag tag	This study
YCplac111-P*REA1*-GFP-*REA1*	CEN, *LEU2*, P*REA1*, T*REA1*, N-terminal GFP tag	[5]
YCplac111-P*REA1*-GFP-*rea1* DAA	CEN, *LEU2*, P*REA1*, T*REA1*, N-terminal GFP tag	[5]
YCplac111-P*REA1*-GFP-*rea1* ΔMIDAS	CEN, *LEU2*, P*REA1*, T*REA1*, N-terminal GFP tag	This study
YCplac111-P*REA1*-GFP-*rea1* Δloop	CEN, *LEU2*, P*REA1*, T*REA1*, N-terminal GFP tag	This study
YCplac111-P*ADH1*-*Sc*MIDAS loop-3xGFP	CEN, *LEU2*, P*ADH1*, T*ADH1*, C-terminal 3xGFP tag	This study
YCplac111-P*ADH1*-*Ct*MIDAS loop-3xGFP	CEN, *LEU2*, P*ADH1*, T*ADH1*, C-terminal 3xGFP tag	This study
YCplac111-P*ADH1*-3xGFP	CEN, *LEU2*, P*ADH1*, T*ADH1*, C-terminal 3xGFP tag	[1]
YCplac111-P*ADH1*-*Ct*MIDAS loop PY>A-3xGFP	CEN, *LEU2*, P*ADH1*, T*ADH1*, C-terminal 3xGFP tag	This study
YCplac111-P*ADH1*-*Ct*MIDAS loop KK>A-3xGFP	CEN, *LEU2*, P*ADH1*, T*ADH1*, C-terminal 3xGFP tag	This study
YCplac111-P*ADH1*-*Ct*MIDAS loop PY/KK>A-3xGFP	CEN, *LEU2*, P*ADH1*, T*ADH1*, C-terminal 3xGFP tag	This study
YCplac111-P*ADH1*-*Sc*MIDAS loop PY>A-3xGFP	CEN, *LEU2*, P*ADH1*, T*ADH1*, C-terminal 3xGFP tag	This study
YCplac111-P*ADH1*-*Sc*MIDAS loop KR>A-3xGFP	CEN, *LEU2*, P*ADH1*, T*ADH1*, C-terminal 3xGFP tag	This study
YCplac111-P*ADH1*-*Sc*MIDAS loop PY/KR>A-3xGFP	CEN, *LEU2*, P*ADH1*, T*ADH1*, C-terminal 3xGFP tag	This study
pG4BDN22-aa4608-*Sc*MIDAS loop>L4-PY-NLS	CEN, *TRP1*, P*ADH1*, T*ADH1*, N-terminal GAL4-BD	This study
pG4BDN22-aa4608-*Sc*MIDAS loop>Hrp1-PY-NLS	CEN, *TRP1*, P*ADH1*, T*ADH1*, N-terminal GAL4-BD	This study
pG4BDN22-aa4608-*Sc*MIDAS loop>Syo1-PY-NLS	CEN, *TRP1*, P*ADH1*, T*ADH1*, N-terminal GAL4-BD	This study
YCplac111-P*REA1*-GFP-*rea1* loop>L4-PY-NLS	CEN, *LEU2*, P*REA1*, T*REA1*, N-terminal GFP tag	This study
YCplac111-P*REA1*-GFP-*rea1* loop>Syo1-PY-NLS	CEN, *LEU2*, P*REA1*, T*REA1*, N-terminal GFP tag	This study
YCplac111-P*REA1*-GFP-*rea1* loop>Hrp1-PY-NLS	CEN, *LEU2*, P*REA1*, T*REA1*, N-terminal GFP tag	This study
YCplac111-P*REA1*-TAP-Flag-*rea1* loop>L4-PY-NLS	CEN, *LEU2*, P*REA1*, T*REA1*, N-terminal TAP-Flag tag	This study
YCplac111-P*REA1*-TAP-Flag-*rea1* loop>Syo1-PY-NLS	CEN, *LEU2*, P*REA1*, T*REA1*, N-terminal TAP-Flag tag	This study
YCplac111-P*REA1*-TAP-Flag-*rea1* loop>Hrp1-PY-NLS	CEN, *LEU2*, P*REA1*, T*REA1*, N-terminal TAP-Flag tag	This study
YCplac111-P*GAL1-10*-TAP-Flag-*rea1* loop>L4-PY-NLS	CEN, *LEU2*, P*GAL1-10*, T*REA1*, N-terminal TAP-Flag tag	This study
YCplac111-P*GAL1-10*-TAP-Flag-*rea1* loop>Syo1-PY-NLS	CEN, *LEU2*, P*GAL1-10*, T*REA1*, N-terminal TAP-Flag tag	This study
YCplac111-P*GAL1-10*-TAP-Flag-*rea1* loop>Hrp1-PY-NLS	CEN, *LEU2*, P*GAL1-10*, T*REA1*, N-terminal TAP-Flag tag	This study
YCplac111-P*GAL1-10*-GFP-*REA1*	CEN, *LEU2*, P*GAL1-10*, T*REA1*, N-terminal GFP tag	[5]
YCplac111-P*GAL1-10*-GFP-*rea1* DAA	CEN, *LEU2*, P*GAL1-10*, T*REA1*, N-terminal GFP tag	[5]
YCplac111-*PGAL1-10*-GFP-*rea1* loop>Hrp1-PY-NLS	CEN, *LEU2*, P*GAL1-10*, T*REA1*, N-terminal GFP tag	This study
pADH181-ProtA-TEV-NAAA^{+} *Ct*Rea1	2µ, *LEU2*, P*ADH1*, T*ADH1*, N-terminal ProtA-TEV tag (aa 1–2390)	This study

Supplementary Table 3. *S. cerevisiae* strains used in this study

Name	Relevant genotype	Source
PJ69-4A	*trp1-901*, *leu2-3,112*, *ura3-52*, *his3-200*, *gal4Δ*, *gal80Δ*, *LYS2*::*GAL1-HIS3*, *GAL2-ADE2*, *met2*::*GAL7-lacZ*	[7]
W303	Wild type, *MAT*α	[8]
DS1-2b *	Wild type	[9]
***rea1*Δ shuffle**	*rea1*::kanMX6, pRS416-*REA1*	[3]
***rea1*Δ *rsa4*Δ shuffle**	*rea1*::kanMX6, *rsa4*::His3MX6, pRS416-*REA1*, pRS316-*RSA4*	[3]
***rea1*Δ *ytm1*Δ shuffle**	*rea1*::His3MX6, *ytm1*::hphNT1, pRS416-*REA1*, pRS316-*YTM1*	[6]
Aid-HA-*REA1*	P_{REA1}-Aid-HA::natNT2, P_{ADH1}-*Os*TIR1-9xmyc::TRP1	This study
***RIX1*-TAP *RPL3*-Flag ***	*RIX1*-TAP::*TRP1*, *RPL3*-Flag::natNT2	[5]

Supplementary References

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