Supplementary Materials for

Structural and functional analysis of Utp23, a yeast ribosome synthesis factor with degenerate PIN domain

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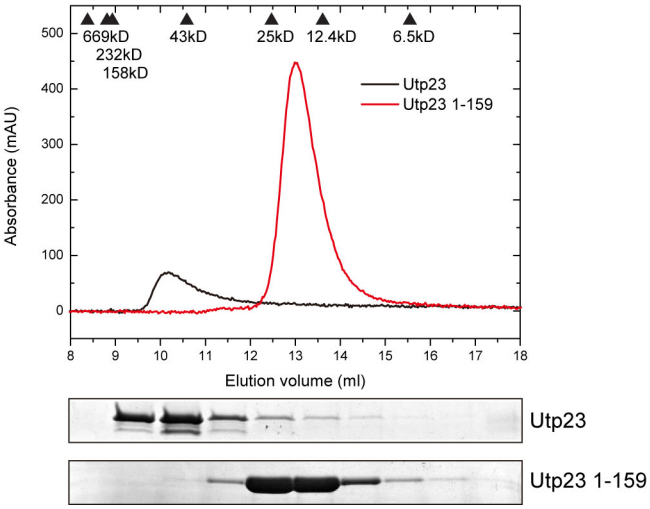

Figure S1. Size exclusion chromatography profiles of full-length Utp23 protein and the PIN domain (1-159). Utp23 1-159 (150 µM) and full-length Utp23 (25 µM) were loaded in 200 µl to a Superdex 75 column. The elution positions of standard proteins thyroglobulin (669kD), catalase (232kD), aldolase (158kD), ovalbumin (43kD), chymotrypsinogen (25kD), cytochrome C (12.4kD) and aprotinin (6.5kD) are indicated on the top.

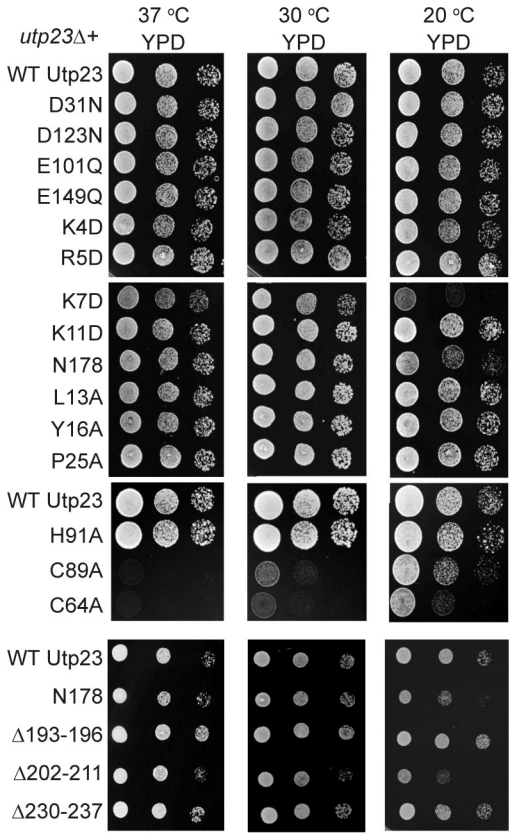

Figure S2. Growth assay of Utp23 variants. The alive *utp23Δ* strains complemented with *LEU* plasmids expressing TAP-tagged Utp23 variants were recovered from 5-FOA plates. Equal amounts of cells were diluted in ten-fold series and grown in YPD plates for 3-4 days at 20 °C and for 2 days at 30 °C and 37 °C.

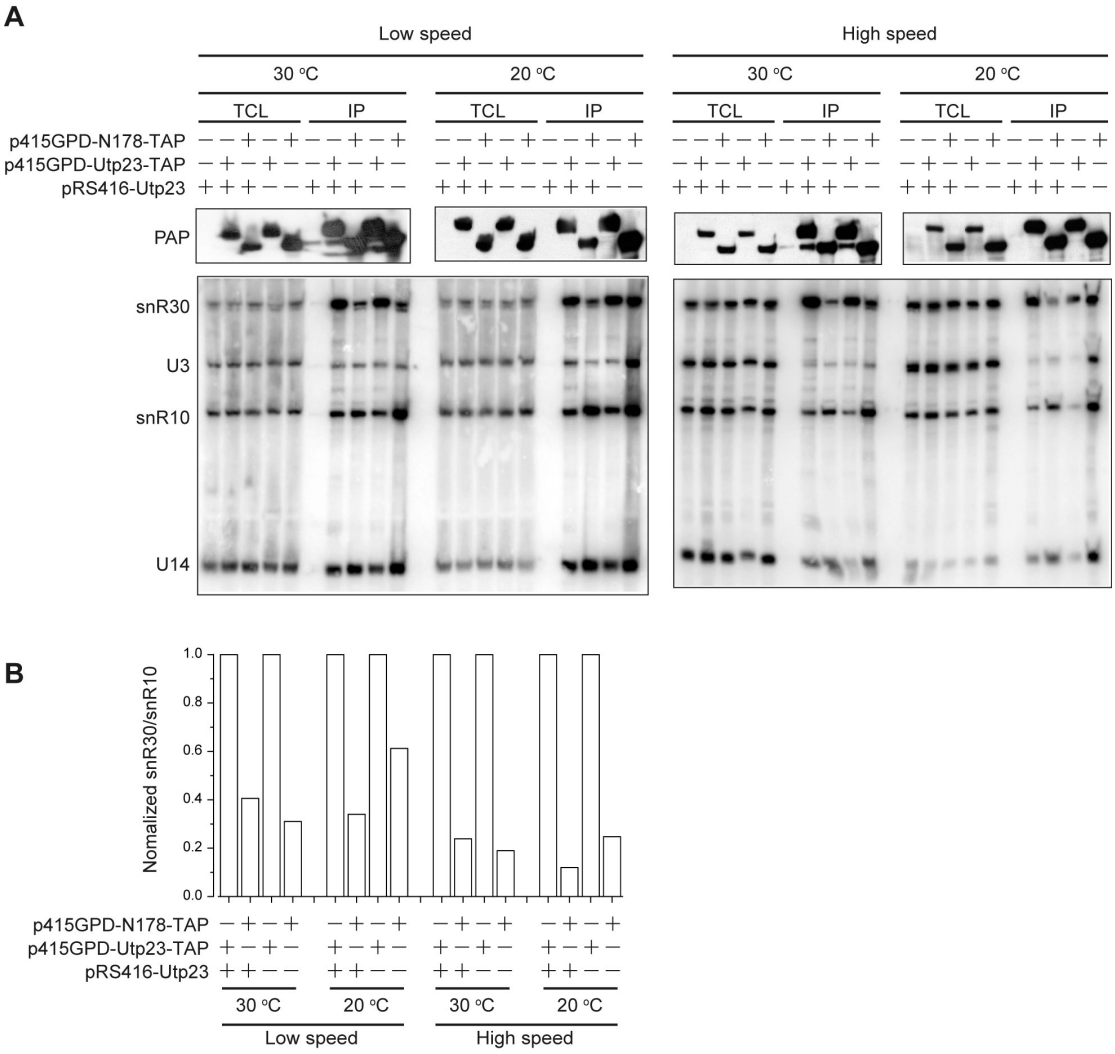

Figure S3. Association of Utp23 with snR30.

(A) The *utp23Δ* strain containing a *URA* plasmid pRS416-UTP23 or not and a *LEU* plasmid expressing TAP-tagged Utp23 or N178 mutant were grown at 30 °C or 20 °C. Cell extracts were centrifuged at 10,000 g for 30 min (low speed) and additionally at 180,000 g for 45 min to deplete large RNPs (high speed). Total cell lysates (TCL, 5%) and immunoprecipitations (IP) of IgG Sepharose were analyzed by western blotting to detect Utp23-TAP and by northern blotting with appropriate DNA probes to detect RNAs. (B) Quantification of snR30. The snR30 to snR10 ratio of the N178 mutant is normalized against that of wild-type Utp23 with the otherwise same treatment.

Table S1.Strain list

Strain	Genotype	Information
BY4741	MATa, his3Δ1, leu2Δ0, met15Δ0, ura3Δ0	Euroscarf
BY4743 UTP23/utp23 Δ::kanMX4	MAT a/α, his3Δ1/his3Δ1, leu2Δ0/leu2Δ0, lys2Δ0/LYS2, MET15/met15Δ0, ura3Δ0/ ura3Δ0, UTP23/utp23Δ::kanMX4	Euroscarf
Utp23 shuffle	MAT a (or α), his3Δ1, leu2Δ0, ura3Δ0, LYS2(or lys2Δ0), MET15(or met15Δ0), utp23Δ::kanMX4, [pRS416-UTP23]	This study; derived from UTP23/utp23Δ

Table S2. Oligonucletide list

Primer name	Sequence
Utp23-pED-F	5’-CAGGGGCCCGAAGCTATGCGTCAAAAGAGGGCTAA-3’
Utp23-pED-R	5’-GCCGCAAGCTGAATTTTACTGCGCGGCTGTGGTC-3’
Utp23N159-QC-F	5’-GCGAGTGCCAAGGCAAGCTAAAATTCAGCTGCGGCCG-3’
Utp23N159-QC-R	5’-CGGCCGCAGCTGAATTTTAGCTTGCCTTGGCACTCGC-3’
Utp23-pRS416-IN-F	5’-CGGTATCGATAAGCTTCTTTGATTTTGAATTTAGG-3’
Utp23-pRS416-IN-R	5’-CGGGCTGCAGGAATTCTGGTGCGTTTTGCGGAGGCT-3’
Utp23-K4D-F	5’-AGTGGATCCATGCGTCAAgAtAGGGCTAAGTCATATAG-3’
Utp23-K4D-R	5’-CTATATGACTTAGCCCTATCTTGACGCATGGATCCACT-3’
Utp23-R5D-QC-F	5’-GATCCATGCGTCAAAAGgacGCTAAGTCATATAGGA-3’
Utp23-R5D-QC-R	5’-TCCTATATGACTTAGCGTCCTTTTGACGCATGGATC-3’
Utp23K4DR5D-GPD-F	5’-AGTGGATCCATGCGTCAAgAtgacGCTAAGTCATATAGGAAA-3’
Utp23K4DR5D-GPD- R	5’-TTTCCTATATGACTTAGCGTCATCTTGACGCATGGATCCACT-3’
Utp23-K7D-F	5’-ATGCGTCAAAAGAGGGCTgAcTCATATAGGAAACAACT-3’
Utp23-K7D-R	5’-AGTTGTTTCCTATATGAGTCAGCCCTCTTTTGACGCAT-3’
Utp23-R10D-F	5’-AGAGGGCTAAGTCATATgatAAACAACTTCTTGTTTA-3’
Utp23-R10D-R	5’-TAAACAAGAAGTTGTTTATCATATGACTTAGCCCTCT-3’
Utp23-K11D-F	5’-AGGGCTAAGTCATATAGGgAcCAACTTCTTGTTT5’-ACAG-3’
Utp23-K11D-R	5’-CTGTAAACAAGAAGTTGGTCCCTATATGACTTA5’-GCCCT-3’
Utp23R10DK11D- F	5’-AAGAGGGCTAAGTCATATgatgAcCAACTTCTTGTTTACAGT-3’
Utp23R10DK11D-R	5’-ACTGTAAACAAGAAGTTGGTCATCATATGACTTAGCCCTCTT-3’
Utp23K4DR5DR10DK11D-F	5’-gAtgacGCTAAGTCATATgatgAcCAACTTCTTGTTTACAGT-3’
Utp23K4DR5DR10DK11D-R	5’-ACTGTAAACAAGAAGTTGGTCATCATATGACTTAGCGTCATC-3’
Utp23-K11A-F	5’-AGGGCTAAGTCATATAGGgcACAACTTCTTGTTTACAG-3’
Utp23-K11A-R	5’-CTGTAAACAAGAAGTTGTGCCCTATATGACTTAGCCCT-3’
Utp23-L13A-F	5’-AGTCATATAGGAAACAAgcTCTTGTTTACAGTCA5’-CAC-3’
Utp23-L13A-R	5’-GTGTGACTGTAAACAAGAGCTTGTTTCCTATATGACT-3’
Utp23-Y16A-F	5’-GGAAACAACTTCTTGTTgcCAGTCACACATTTAAGT-3’
Utp23-Y16A-R	5’-ACTTAAATGTGTGACTGGCAACAAGAAGTTGTTTCC-3’
Utp23-P25A-F	5’-ACATTTAAGTTCCGTGAGgCATATCAAGTTTTGGTTG-3’
Utp23-P25A-R	5’-CAACCAAAACTTGATATGCCTCACGGAACTTAAATGT-3’
Utp23-D31N-F	5’-CCATATCAAGTTTTGGTTaATAATCAGCTTGTTTTAG-3’
Utp23-D31N-R	5’-CTAAAACAAGCTGATTATTAACCAAAACTTGATATGG-3’
Utp23-D123N-F	5’-TATGTGGTTGCTTCTCAGaACATAGATTTGAGGAGGA-3’
Utp23-D123N-F	5’-TCCTCCTCAAATCTATGTTCTGAGAAGCAACCACATA-3’
Utp23-E101Q-F	5’-GATCCAAAGTCACCTGCGcAGTGCATCGAAAGCGTC-3’
Utp23-E101Q-R	5’-GACGCTTTCGATGCACTGCGCAGGTGACTTTGGATC-3’
Utp23-E149Q-F	5’-AGGTCTGTTATGGTTATGcAACCTCTCAGTACGGCG-3’
Utp23-E149Q-F	5’-CGCCGTACTGAGAGGTTGCATAACCATAACAGACCT-3’
Utp23Δ1-23-p415GPD-F	5’-AGAACTAGTGGATCCATGGAGCCATATCAAGTTTTG-3’
Utp23Δ1-23-p415GPD-R	5’-CAAAACTTGATATGGCTCCATGGATCCACTAGTTCT-3’
Utp23-N178-p415GPD-QC-F	5’-GATCCTAATATTGAAAAAGGTGAGTCCATGGAAAAG-3’
Utp23-N178-p415GPD-QC-R	5’-CTTTTCCATGGACTCACCTTTTTCAATATTAGGATC-3’
Utp23N159-GPD-QC-F	5’-GCGAGTGCCAAGGCAAGCGGTGAGTCCATGGAAAAG-3’
Utp23N159-GPD-QC-R	5’-CTTTTCCATGGACTCACCGCTTGCCTTGGCACTCGC-3’
Utp23-C64A-F	5’-AGGTCATGATCACACAGgcTTGCATACAAGCGTTATA-3’
Utp23-C64A-R	5’-TATAACGCTTGTATGCAAgcCTGTGTGATCATGACCT-3’

Utp23-C89A-F	5’-CAATTTGAGAGACGTCGTgcTAACCACTCTTTTAAAGA-3’
Utp23-C89A-R	5’-TCTTTAAAAGAGTGGTTAgcACGACGTCTCTCAAATTG-3’
Utp23-H91A-F	5’-GAGAGACGTCGTTGTAACgcCTCTTTTAAAGATCCAAAG-3’
Utp23-H91A-R	5’-CTTTGGATCTTTAAAAGAGGCGTTACAACGACGTCTCTC-3’
Utp23-C102A-F	5’-CAAAGTCACCTGCGGAGgcCATCGAAAGCGTCGTTAA-3’
Utp23-C102A-R	5’-TTAACGACGCTTTCGATGgcCTCCGCAGGTGACTTTG-3’
Utp23-Del193-196-F	5’-GGAAAGGAATCTATCACTCTTGGTCCAAAGGCTCCT-3’
Utp23-Del193-196-R	5’-AGGAGCCTTTGGACCAAGAGTGATAGATTCCTTTCC-3’
Utp23-Del202-211-F	5’-AAGCTTGGTCCAAAGGCTAAAGTCAACTCGCCCAGC -3’
Utp23-Del202-211-R	5’-GCTGGGCGAGTTGACTTTAGCCTTTGGACCAAGCTT-3’
Utp23-Del230-237-F	5’-GAAGATACTTCTAAAGAACACAAAAGCAACACTAAC-3’
Utp23-Del230-237-R	5’-GTTAGTGTTGCTTTTGTGTTCTTTAGAAGTATCTTC-3’

Table S3. Plasmid list

Plasmids	Features	Information
pETDuet-Utp23	Full-length Utp23 with an N-terminal His6-tag followed by a PreScission cleavage site	The Utp23 gene was PCR amplified from genomic DNA with primers Utp23-pED-F and Utp23-pED-R and inserted by In-fusion into pETDuet-HS linearized with HindIII and EcoRI.
pETDuet-Utp23 1-159	Utp23 1-159 with an N-terminal cleavable His6-tag.	pETDuet-Utp23 was mutagenized by QuikChange with primers Utp23N159-QC-F and Utp23N159-QC-R.
pRS416	CEN; URA3	Brachmann et al 1998
pRS416-Utp23	CEN; URA3; Utp23 ORF including 700 nt before start codon and 188 nt after stop codon	The Utp23 gene was PCR amplified from genomic DNA with primers Utp23-pRS416-IN-F and Utp23-pRS416-IN-R and inserted by In-fusion into pRS416 linearized with HindIII and EcoRI.
p415GPD-Utp23-TAP	CEN; LEU2; GPD promoter; Utp23-TAP fusion	provided by Dr. Susan Baserga
p415GPD-Utp23K4D-TAP	p415GPD-Utp23-TAP with Utp23 mutation K4D	p415GPD-Utp23-TAP was mutagenized by QuikChange with primers Utp23-K4D-F and Utp23-K4D-R.
p415GPD-Utp23R5D-TAP	p415GPD-Utp23-TAP with Utp23 mutation R5D	p415GPD-Utp23-TAP was mutagenized by QuikChange with primers Utp23-R5D-QC-F and Utp23-R5D-QC-R.
p415GPD-Utp23K7D-TAP	p415GPD-Utp23-TAP with Utp23 mutation K7D	p415GPD-Utp23-TAP was mutagenized by QuikChange with primers Utp23-K7D-F and Utp23-K7D-R.
p415GPD-Utp23R10D-TAP	p415GPD-Utp23-TAP with Utp23 mutation R10D	p415GPD-Utp23-TAP was mutagenized by QuikChange with primers Utp23-R10D-F and Utp23-R10D-R.
p415GPD-Utp23K11D-TAP	p415GPD-Utp23-TAP with Utp23 mutation K11D	p415GPD-Utp23-TAP was mutagenized by QuikChange with primers Utp23-K11D-F and Utp23-K11D-R.
p415GPD-Utp23K4DR5D-TAP	p415GPD-Utp23-TAP with Utp23 mutation K4DR5D	p415GPD-Utp23-TAP was mutagenized by QuikChange with primers Utp23K4DR5D-GPD-F and Utp23K4DR5D-GPD-R.
p415GPD-Utp23R10DK11D-TAP	p415GPD-Utp23-TAP with Utp23 mutation R10DK11D	p415GPD-Utp23-TAP was mutagenized by QuikChange with primers Utp23R10DK11D-F and Utp23R10DK11D-R.
p415GPD-Utp23K4DR5DR10DK11D-TAP	p415GPD-Utp23-TAP with Utp23 mutation K4DR5DR10DK11D	p415GPD-Utp23K4DR5D-TAP was mutagenized by QuikChange with primers Utp23K4DR5D R10DK11D-F and Utp23K4DR5D R10DK11D-R.
p415GPD-Utp23L13A-TAP	p415GPD-Utp23-TAP with Utp23 mutation L13A	p415GPD-Utp23-TAP was mutagenized by QuikChange with primers Utp23- L13A -F and Utp23- L13A -R.
p415GPD-Utp23Y16A-TAP	p415GPD-Utp23-TAP with Utp23 mutation Y16A	p415GPD-Utp23-TAP was mutagenized by QuikChange with primers Utp23-Y16A-F and Utp23-Y16A-R.
p415GPD-Utp23P25A-TAP	p415GPD-Utp23-TAP with Utp23 mutation P25A	p415GPD-Utp23-TAP was mutagenized by QuikChange with primers Utp23-P25A-F and Utp23-P25A-R.
p415GPD-Utp23Δ1-23-TAP	p415GPD-Utp23-TAP with deletion of Utp23 residues 1-23	p415GPD-Utp23-TAP was mutagenized by QuikChange with primers Utp23Δ1-23-p415GPD-F and Utp23Δ1-23-p415GPD-R.

p415GPD- Utp23 1-159(N159)-TAP	p415GPD-Utp23-TAP with deletion of Utp23 residues 160-254	p415GPD-Utp23-TAP was mutagenized by QuikChange with primers Utp23N159-GPD-QC-F and Utp23N159-GPD-QC-R.
p415GPD-Utp23 1-178 (N178)-TAP	p415GPD-Utp23-TAP with deletion of Utp23 residues 179-254	p415GPD-Utp23-TAP was mutagenized by QuikChange with primers Utp23-N178-p415GPD-QC-F and Utp23-N178-p415GPD-QC-R.
p415GPD-Utp23D31N-TAP	p415GPD-Utp23-TAP with Utp23 mutation D31N	p415GPD-Utp23-TAP was mutagenized by QuikChange with primers Utp23-D31N-F and Utp23-D31N-R.
p415GPD-Utp23D123N-TAP	p415GPD-Utp23-TAP with Utp23 mutation D123N	p415GPD-Utp23-TAP was mutagenized by QuikChange with primers Utp23-D123N-F and Utp23-D123N-R.
p415GPD-Utp23E101Q-TAP	p415GPD-Utp23-TAP with Utp23 mutation E101Q	p415GPD-Utp23-TAP was mutagenized by QuikChange with primers Utp23-E101Q-F and Utp23-E101Q-R.
p415GPD-Utp23E149Q-TAP	p415GPD-Utp23-TAP with Utp23 mutation E149Q	p415GPD-Utp23-TAP was mutagenized by QuikChange with primers Utp23-E149Q-F and Utp23-E149Q-R.
p415GPD-Utp23C64A-TAP	p415GPD-Utp23-TAP with Utp23 mutation C64A	p415GPD-Utp23-TAP was mutagenized by QuikChange with primers Utp23-C64A-F and Utp23-C64A-R.
p415GPD-Utp23C89A-TAP	p415GPD-Utp23-TAP with Utp23 mutation C89A	p415GPD-Utp23-TAP was mutagenized by QuikChange with primers Utp23-C89A-F and Utp23-C89A-R.
p415GPD-Utp23H91A-TAP	p415GPD-Utp23-TAP with Utp23 mutation H91A	p415GPD-Utp23-TAP was mutagenized by QuikChange with primers Utp23-H91A-F and Utp23-H91A-R.
p415GPD-Utp23C102A-TAP	p415GPD-Utp23-TAP with Utp23 mutation C102A	p415GPD-Utp23-TAP was mutagenized by QuikChange with primers Utp23- C102A -F and Utp23- C102A -R.
p415GPD-Utp23Δ193-196-TAP	p415GPD-Utp23-TAP with deletion of Utp23 residues 193-196	p415GPD-Utp23-TAP was mutagenized by QuikChange with primers Utp23-Del193-196-F and Utp23-Del193-196-R.
p415GPD-Utp23Δ202-211-TAP	p415GPD-Utp23-TAP with deletion of Utp23 residues 202-211	p415GPD-Utp23-TAP was mutagenized by QuikChange with primers Utp23-Del202-211-F and Utp23-Del202-211-R.
p415GPD-Utp23Δ230-237-TAP	p415GPD-Utp23-TAP with deletion of Utp23 residues 230-237	p415GPD-Utp23-TAP was mutagenized by QuikChange with primers Utp23-Del230-237-F and Utp23-Del230-237-R.