Supplementary material

Supplementary Table 1: Yeast strains used in this study

Strain	Genotype	Source
SEY6210	*MATα ura3 leu2 his3 trp1 lys2 suc2-Δ9*	P. Cosson
PC137	*MATa ura3-1 leu2-Δ1 his4-619 trp1-Δ9 lys2-801am suc2-Δ9 tip20-5*	P. Cosson
tip20-8	*MATα ura3 leu2 his4 trp1 lys2 suc2-Δ9 tip20-8*	P. Cosson
YAS2793	*MATα ura3 leu2 his3 trp1 lys2 suc2-Δ9 TIP20::kanMX4 pRS316-TIP20-URA3*	this study
YAS2794	*MATα ura3 leu2 his3 trp1 lys2 suc2-Δ9 TIP20::kanMX4 pRS315-TIP20-LEU2*	this study
YAS2795	*MATα ura3 leu2 his3 trp1 lys2 suc2-Δ9 TIP20::kanMX4 pRS315-tip20F248L-LEU2*	this study
YAS2796	*MATα ura3 leu2 his3 trp1 lys2 suc2-Δ9 TIP20::kanMX4 pRS315-tip20N285S-LEU2*	this study
YAS2797	*MATα ura3 leu2 his3 trp1 lys2 suc2-Δ9 TIP20::kanMX4 pRS315-tip20S563P-LEU2*	this study
YAS2798	*MATα ura3 leu2 his3 trp1 lys2 suc2-Δ9 TIP20::kanMX4 pRS315-tip20F621S-LEU2*	this study
YAS2799	*MATα ura3 leu2 his3 trp1 lys2 suc2-Δ9 TIP20::kanMX4 pRS315-tip20K381E-LEU2*	this study
YAS2800	*MATα ura3 leu2 his3 trp1 lys2 suc2-Δ9 TIP20::kanMX4 pRS315-tip20L435S-LEU2*	this study
YAS2801	*MATα ura3 leu2 his3 trp1 lys2 suc2-Δ9 TIP20::kanMX4 pRS315-tip20Δ1-81-LEU2*	this study
YAS2802	*MATα ura3 leu2 his3 trp1 lys2 suc2-Δ9 TIP20::kanMX4 pRS315-tip20I10DL28E-LEU2*	this study
YAS2803	*MATα ura3 leu2 his3 trp1 lys2 suc2-Δ9 TIP20::kanMX4 pRS315-tip20V17E-LEU2*	this study
YAS2804	*MATα ura3 leu2 his3 trp1 lys2 suc2-Δ9 TIP20::kanMX4 pRS315-TIP20-3HA-LEU2-TRP1*	this study
YAS2805	*MATα ura3 leu2 his3 trp1 lys2 suc2-Δ9 TIP20::kanMX4 pRS315-tip20Δ1-81-3HA-LEU2-TRP1*	this study
YAS2806	*MATα ura3 leu2 his3 trp1 lys2 suc2-Δ9 TIP20::Tip20-STREP-kanMX6*	this study
YAS2807	*MATa ura3-1 leu2-Δ1 his4-619 trp1-Δ9 lys2-801am suc2-Δ9 tip20-5::tip20-5-STREP-kanMX6*	this study
YAS2808	*MATα ura3 leu2 his4 trp1 lys2 suc2-Δ9 tip20-8::tip20-8-STREP-kanMX6*	this study
YAS2809	*MATα ura3 leu2 his3 trp1 lys2 suc2-Δ9 SEC20::Sec20-HBH-TRP1*	this study
YAS2810	*MATa ura3-1 leu2-Δ1 his4-619 trp1-Δ9 lys2-801am suc2-Δ9 tip20-5 SEC20::Sec20-HBH-TRP1*	this study
YAS2811	*MATα ura3 leu2 his4 trp1 lys2 suc2-Δ9 tip20-8 SEC20::Sec20-HBH-TRP1*	this study

Supplementary Table 2: Primers used in this study

Name	Sequence
MDSeq1 (tip20Cterm raus)	TTG AGC TTA CCG TCA CAT GC
MDSeq2 (tip20Nterm raus)	TGC AGT TTT GAT GCC AGT TC
MDSeq3 (tip20part1 left)	AAA ATT GTA GGC AGA AGT AGA TAA GAA
MDSeq4 (tip20part1 right)	TTG AGG AAC TGT AGC GAA GAC A
MDSeq5 (tip20part2 left)	TTT GAA CGT CAG TTA GAT GAG TTA GC
MDSeq6 (tip20part2 right)	CTG GCT TCG TCA ACC CAT T
MDSeq7 (tip20part3 left)	AGG TTT ACG TAC CAT TTT CAC G
MDSeq8 (tip20part3 right)	GCT CTT TAG TTC TTG TTT CAT TCA A
MDSeq9 (tip20part4 left)	GAT TAT TTG GAA CCG TTC TAC GA
MDSeq10 (tip20part4 right)	TAA CAT TTA ACA ATT CGT TCT TAA CC
MDSeq11 (tip20part5 left)	CAA GCG CTG AGT TGG TCA AT
MDSeq12 (tip20part5 right)	TGG TCT GGA GTT ACA TTT GGA
MDTIP1 (Tip20-BamHIfwd)	CGC GGA TCC GCG AGC AGC AAC GAG CGT TTA AT
MDTIP2 (Tip20-BamHIrev)	CGC GGA TCC GCG GCG CCA CAA AAG TTT CCT AC
MDTIP3 (Tip20Dfwd)	TTG TAT ATT AAG TTA TTG TTT ATA AGC ATA GTC ACA AGT GCA TAA CAG CTG AAG CTT CGT ACG C
MDTIP4 (Tip20DeletionS2)	TAA TAC TCG TCT TGT TGA TTT TTT TCC TTC TCT TTT TTT TAC GAG GCA TAG GCC ACT AGT GGA TCT G
MDTIP7 (T20-5UTR_fwd)	CGC TCT AGA TGC CTG CAT TAA ACA CGG TA
MDTIP8 (T20-5UTR_rev)	CCC GGG TAT ATA GGA TCC AGT TTT TAT GCA CTT GTG ACT ATG C
MDTIP9 (T20-3UTR_fwd)	GGA TCC TAT ATA CCC GGG CTC GTA AAA AAA AGA GAA GGA AAA AAA TC
MDTIP10 (T20-3UTR_rev)	CGC CTG CAG TGC AGG AAA GAA AGA ACG TG
MDTIP11 (ARATIforward(BamH1))	CGC GGA TCC ATG AAC GGC ATT GAT GAT CTC
MDTIP12 (ARATIreverse(Kpn1))	CGG GGT ACC TTA TAA TAT GTT ACC ATA TAT AAT CCT ATA G

Supplementary Table 3: Constructs used in this study

construct	plasmid	source
C-terminal GST-tagged cytoplasmic region of Ufe1p (1-327)	pETGEXCT (Sharrocks, 1994)	R. Schekmann (University of California, Department of Molecular and Cell Biology,Berkeley, USA)
N-terminal His_6-tagged cytoplasmic region of Use1p (1-217) co-expressed with Dsl3p	pQLink vector system (Scheich *et al.*, 2007)	F. M. Hughson (Princeton University, Department of Molecular Biology, Princeton, USA)
N-terminal GST-tagged cytoplasmic region of Sec20p (1-275)	pGEXTT	R.-W. Peng (ETH Zürich,DBSSE,Basel, Switzerland).
N-terminal His_6-tagged cytoplasmic region of Sec20p (1 -275)	pQE30	H. D. Schmitt (Max-Planck-Institute for Biophysical Chemistry, Department of Neurobiology, Göttingen, Germany)
C-terminal His_6-tagged cytoplasmic region of Sec22p (1-180)	pET24b	D. K. Banfield (Hong Kong University of Science and Technology, Department of Biology, People's Republic of China)
C-terminal His_6-tagged cytoplasmic region of Bet1p (1-123)	pET24(+)	H. D. Schmitt
C-terminal His_6-tagged cytoplasmic region of Bos1p (1-216)	pET24b	D. K. Banfield
N-terminal His_6-tagged cytoplasmic region of Snc1p (1-93)	pTrcHisC	J. E. Gerst (Weizmann Institute of Science, Department of Molecular Genetics,Israel)
C-terminal His_6-tagged cytoplasmic region of Ykt6p (2-190)	pET24b	D. K. Banfield
N-terminal GST-tagged Dsl1p	pGEXTT	H. D. Schmitt
N-terminal His_6-tagged Dsl1p	pProExHTb	F. M. Hughson
N-terminal His_6-tagged Tip20p	pProExHTb	F. M. Hughson
N-terminal His_6-tagged Tip20-5p	pProExHTb	this study
N-terminal His_6-tagged Tip20-8p		

Supplementary Figure legends

Figure S1

Evolutionary conservation profile for Tip20p as calculated by the ConSurf Server (Ashkenazy *et al.*, 2010). **(A)** Secondary structure representation of Tip20p. The amino acids are colored by their conservation grade according to the gradient shown, with blue (1) to red (9) indicating variable to conserved. The red triangles indicate the mutations found in Tip20-8p and the green triangles the mutations found in Tip20-5p. The amino acids that occur in the mutations are indicated under the respective triangles. **(B)** Amino acid conservation scores for the residues found to be mutated in Tip20-8p and Tip20-5p. 3LATOM: The ATOM derived sequence in three letter code, including the amino acid positions as they appear in the PDB file and the chain identifier. SCORE: The normalized conservation scores. COLOR: The color scale representing the conservation scores (9: conserved, 1: variable). CONFIDENCE INTERVAL: A confidence interval is assigned to each of the inferred evolutionary conservation scores. CONFIDENCE INTERVAL COLORS: The color scale representing the lower and upper bounds of the confidence interval. MSA DATA: The number of aligned sequences having an amino acid (non-gapped) from the overall number of sequences at each position. RESIDUE VARIETY: The residue variety at each position of the multiple sequence alignment.

Figure S2

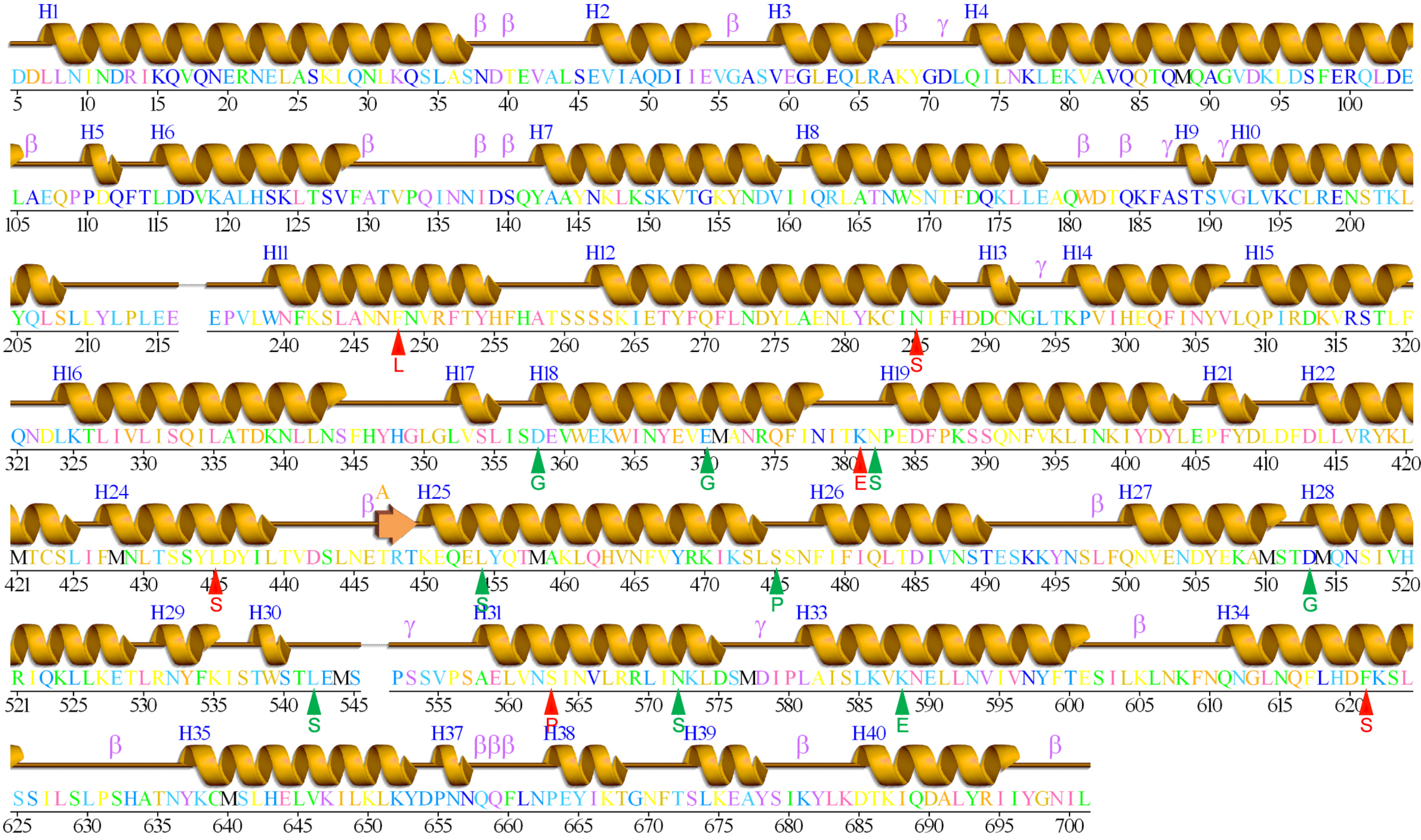
Tip20-5p and Tip20-8p behave similar to Tip20p in a differential centrifugation after a shift of the respective strains to 37°C for 1 hr. Logarithmically growing strains were shifted to 37°C for an hour before subjecting them to cell lysis and differentially centrifugation. The pellets of the centrifugation were resuspended to the same volume than the final supernatant and equal volumes were loaded onto an SDS PAGE followed by immunoblot analysis. All Tip20p variants behaved very similar, again indicating that the Tip20p mutant proteins do not aggregate.

Figure S3

Tip20p binds directly to Ufe1p. **(A)** A GST pulldown was performed with GST-Ufe1p and the Tip20p variants. **(B)** Tip20p binds with low affinity to GST-Ufe1p, but does not aggregate on the beads. Increasing amounts (2.25 µg to 40.5 µg) of His-Tip20 were incubated with 5 µg GST-Ufe1, immobilized onto GSH agarose beads. His-Tip20p bound specifically but not with a high affinity. The input represents 1.13 µg of His-Tip20p. These data also proof that Tip20p does not form aggregates on the beads. **(C)** His-Tip20p does not bind to GST. A similar pull down was performed as described in (B). In parallel, two independent titration pull-downs were performed with GST. While His-Tip20 binds still to GST-Ufe1p, no binding to GST was observed under the same conditions.

Supplementary figure 1

A

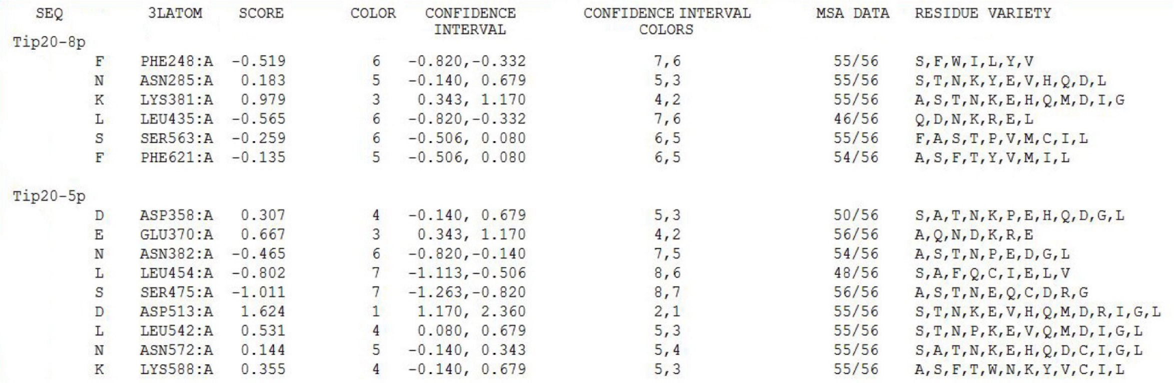


Sec. struc.: Helix Strand Helices labeled H1, H2 ... and strands by their sheets A, B ...

Motifs: β beta turn, γ gamma turn

Conservation colouring: **Low 1 2 3 4 5 6 7 8 9 High**

B



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  SEQ             3LATOM     SCORE          COLOR   CONFIDENCE          CONFIDENCE INTERVAL         MSA DATA    RESIDUE VARIETY
                                                      INTERVAL                 COLORS
 Tip20-8p
           F     PHE248:A   -0.519             6    -0.820,-0.332              7,6                   55/56     S,F,W,I,L,Y,V
           N     ASN285:A    0.183             5    -0.140, 0.679              5,3                   55/56     S,T,N,K,Y,E,V,H,Q,D,L
           K     LYS381:A    0.979             3     0.343, 1.170              4,2                   55/56     A,S,T,N,K,E,H,Q,M,D,I,G
           L     LEU435:A   -0.565             6    -0.820,-0.332              7,6                   46/56     Q,D,N,K,R,E,L
           S     SER563:A   -0.259             6    -0.506, 0.080              6,5                   55/56     F,A,S,T,P,V,M,C,I,L
           F     PHE621:A   -0.135             5    -0.506, 0.080              6,5                   54/56     A,S,F,T,Y,V,M,I,L

 Tip20-5p
           D     ASP358:A    0.307             4    -0.140, 0.679              5,3                   50/56     S,A,T,N,K,P,E,H,Q,D,G,L
           E     GLU370:A    0.667             3     0.343, 1.170              4,2                   56/56     A,Q,N,D,K,R,E
           N     ASN382:A   -0.465             6    -0.820,-0.140              7,5                   54/56     A,S,T,N,P,E,D,G,L
           L     LEU454:A   -0.802             7    -1.113,-0.506              8,6                   48/56     S,A,F,Q,C,I,E,L,V
           S     SER475:A   -1.011             7    -1.263,-0.820              8,7                   56/56     A,S,T,N,E,Q,C,D,R,G
           D     ASP513:A    1.624             1     1.170, 2.360              2,1                   55/56     S,T,N,K,E,V,H,Q,M,D,R,I,G,L
           L     LEU542:A    0.531             4     0.080, 0.679              5,3                   55/56     S,T,N,P,K,E,V,Q,M,D,I,G,L
           N     ASN572:A    0.144             5    -0.140, 0.343              5,4                   55/56     S,A,T,N,K,E,H,Q,D,C,I,G,L
           K     LYS588:A    0.355             4    -0.140, 0.679              5,3                   55/56     A,S,F,T,W,N,K,Y,V,C,I,L
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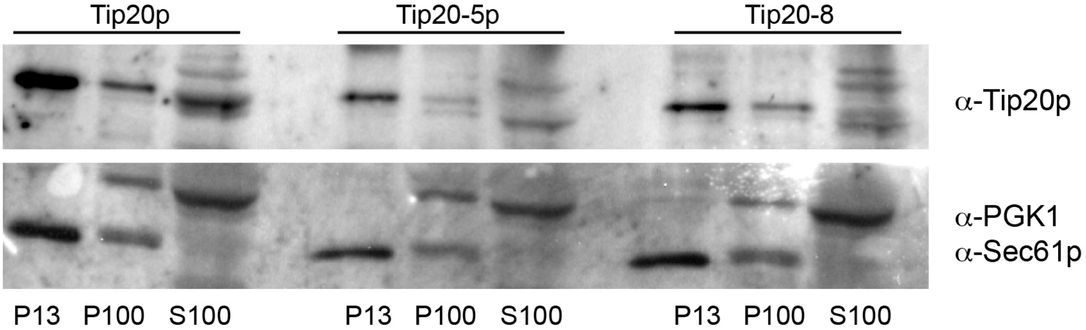
Supplemental Figure 2



Supplementary Figure 3