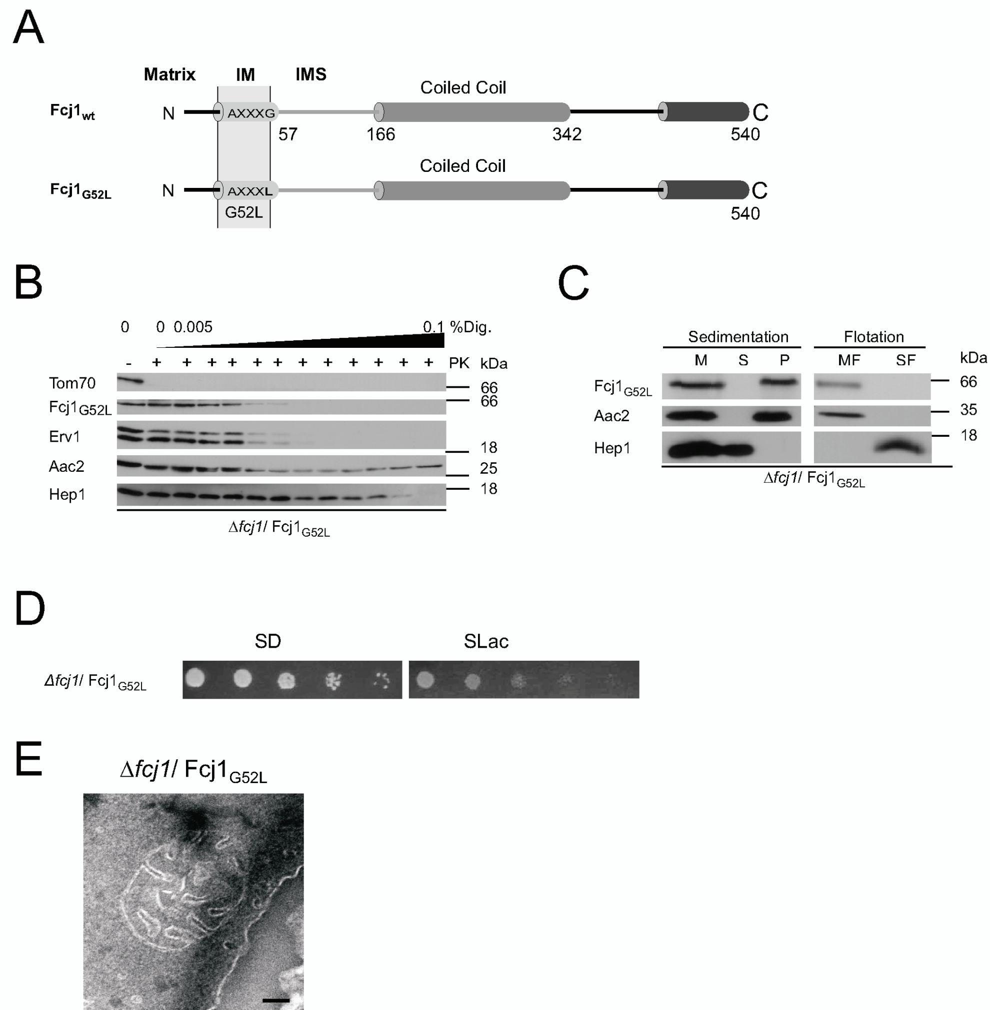
Supplementary information

Supplementary materials and methods

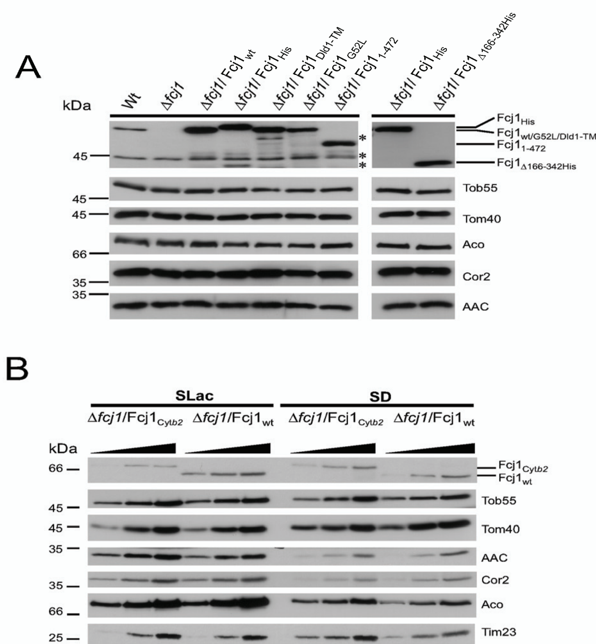
Construction of Fcj1 variants

All Fcj1 variants were cloned into the pYX242 vector and transformed into Δ*fcj1* (W303α) strain (Rabl *et al.*, 2009). Cloning of Fcj1wt and Fcj1His are described in earlier study (Rabl *et al.*, 2009). $Fcj1_{1\text{-}472}$ was generated by amplifying the Fcj1 gene corresponding to amino acids 1-472 using the Fcj1fo and $Fcj1_{472rev}$ primers and cloning the generated fragment into pYX242 using NcoI/XhoI restrictions sites. For generation of $Fcj1_{\Delta 166\text{-}342His}$ the fragments $Fcj1_{1\text{-}165}$ and $Fcj1_{343\text{-}540His}$ were made by amplification using the primers Fcj1fo/Fcj1dCCrev and Fcj1dCCfo/Fcj1His12rev respectively; fragments were cloned into pYX242 using NcoI-BamHI and BamHI-XhoI restriction sites, respectively. For cloning $Fcj1_{DLD1\text{-}TM}$, two fragments were amplified using primers Fcj1fo/Fcj1DLDrev and Fcj1DLDfo/Fcj1rev, respectively. Fcj1DLD was amplified by fusion PCR using two generated fragments as template and Fcj1fo/Fcj1rev as primers and cloned into pYX242 vector using NcoI/XhoI restriction sites. $Fcj1_{G52L}$ was generated by amplification of two fragments using Fcj1up/Fcj1G52Lrev and Fcj1G52Lfo/Fcj1rev primers, respectively. Fragments were sequentially cloned into pYX242 vector using NcoI/AvrI and AvrI/XhoI restriction sites. For generation of $Fcj1Cyt_{b2}$, fragment $Cytb_2$1-167 was subcloned from pYX142 $Cyt\textit{b2}_{1\text{-}167}$-$Tim50_{132\text{-}476}$ vector into pYX242 vector using EcoRI/BamHI restriction sites. Fragment $Fcj1_{57\text{-}540}$ was amplified by using Fcj1N57fo and Fcj1rev primers and genomic yeast DNA as template and cloned into pYX242Cyt$b_2$1-167 using BamHI/XhoI restriction sites. $Fcj1_{1\text{-}472His}$ was generated by amplifying the fragment $Fcj1_{1\text{-}472His}$ using the primers Fcj1fo and Fcj1472Hisrev and cloning the generated fragment into pYX242 using NcoI/XhoI restrictions sites.

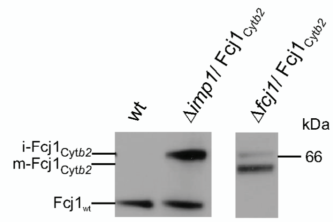
Supplementary figure legends



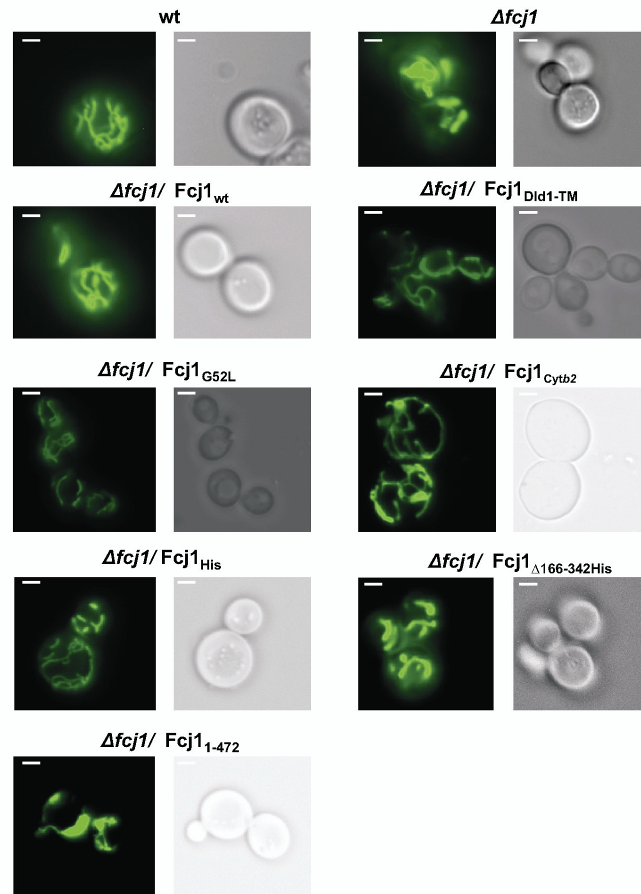
Supplementary Figure S1. **Putative dimerization motif in Fcj1 is not important for Fcj1 function. A,** Schematic representation of Fcj1 wild type ($Fcj1_{wt}$) and the dimerisation mutant ($Fcj1_{G52L}$) with mutation Gly 52 to Leu. **B,** Submitochondrial localization of $Fcj1_{G52L}$ variant was performed as described in Fig. 1B. **C,** Membrane insertion of $Fcj1_{G52L}$ variant was determined as described in Fig. 1C. **D,** Expression of $Fcj1_{G52L}$ can rescue the growth phenotype of Δ*fcj1* on non-fermentable carbon source (SLac). Drop dilution assay was performed as described in Fig. 1D. Corresponding controls from the same agar plate and the same digital image are shown in Fig. 1D, top panels. **E,** Electron micrographs of mitochondria, as in Figure 1E.

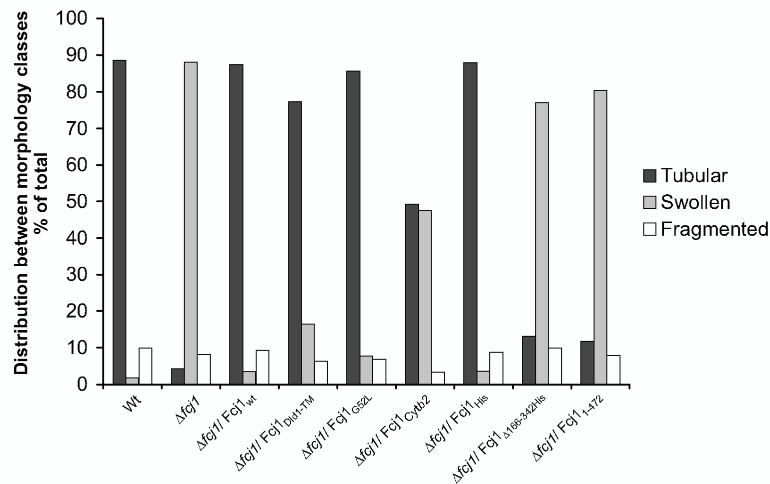


Supplementary Figure S2. **Protein levels of Fcj1 variants and mitochondrial marker proteins. A,** Mitochondria from the indicated strains were subjected to SDS-PAGE and proteins were analyzed by immunblotting with indicated antibodies. Asterisks, unspecific cross reactions and/or degradation products. **B,** Mitochondria from $Fcj1_{wt}$ and $Fcj1_{Cyt\mathit{b2}}$ strains grown on either fermentable (SD) or non-fermentable (SLac) selective medium were analyzed as in A. Increasing amounts (6.25, 12.5 and 25 μg) were loaded as indicated.



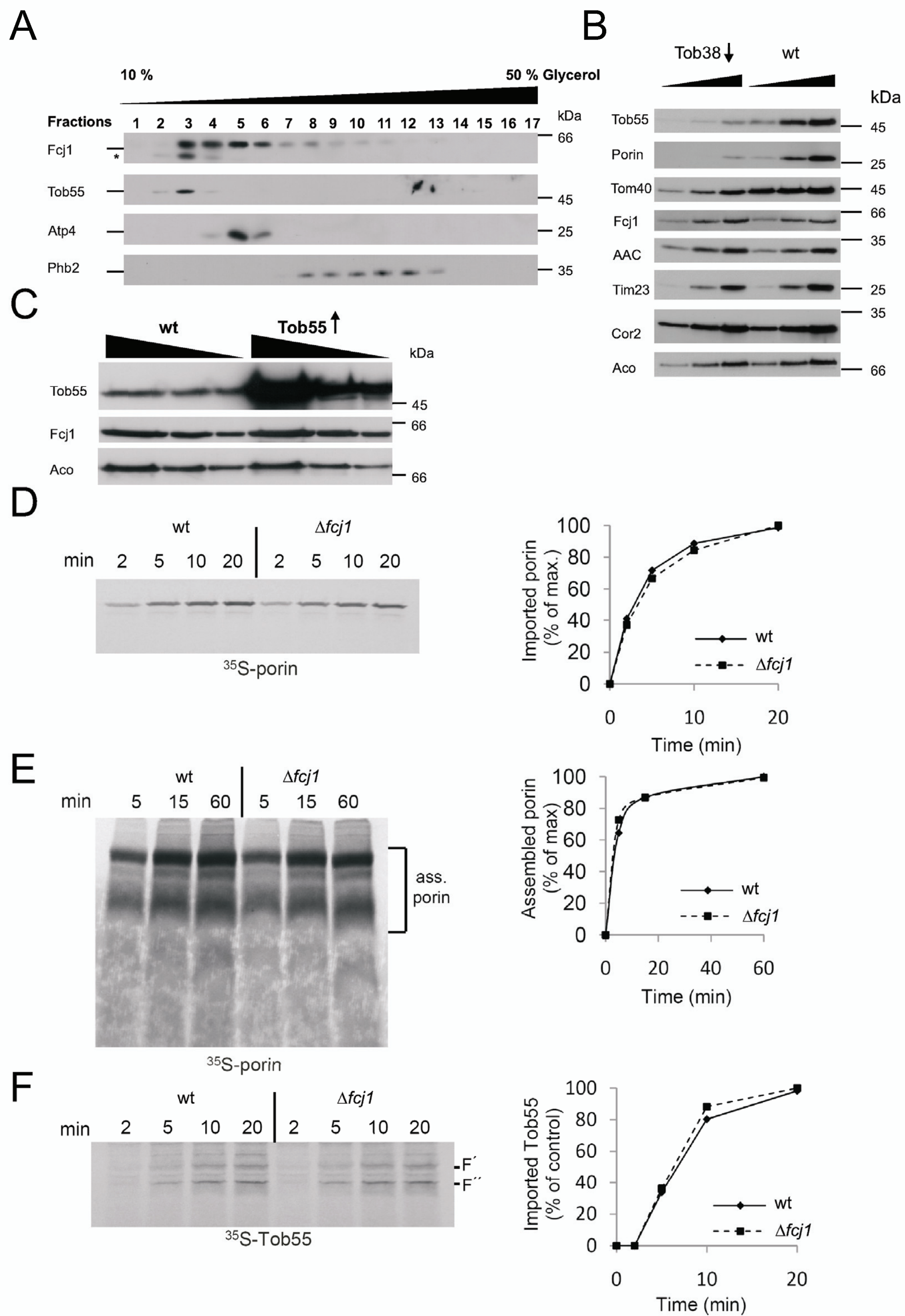
Supplementary Figure S3. **Fcj1$_{Cyt\mathit{b2}}$ is cleaved by Imp1.** Equal amounts of cell lysate from the indicated strains were subjected to SDS-PAGE and proteins were analyzed by immunodecoration for Fcj1. The intermediate (i) and mature (m) forms of Fcj1 are indicated.



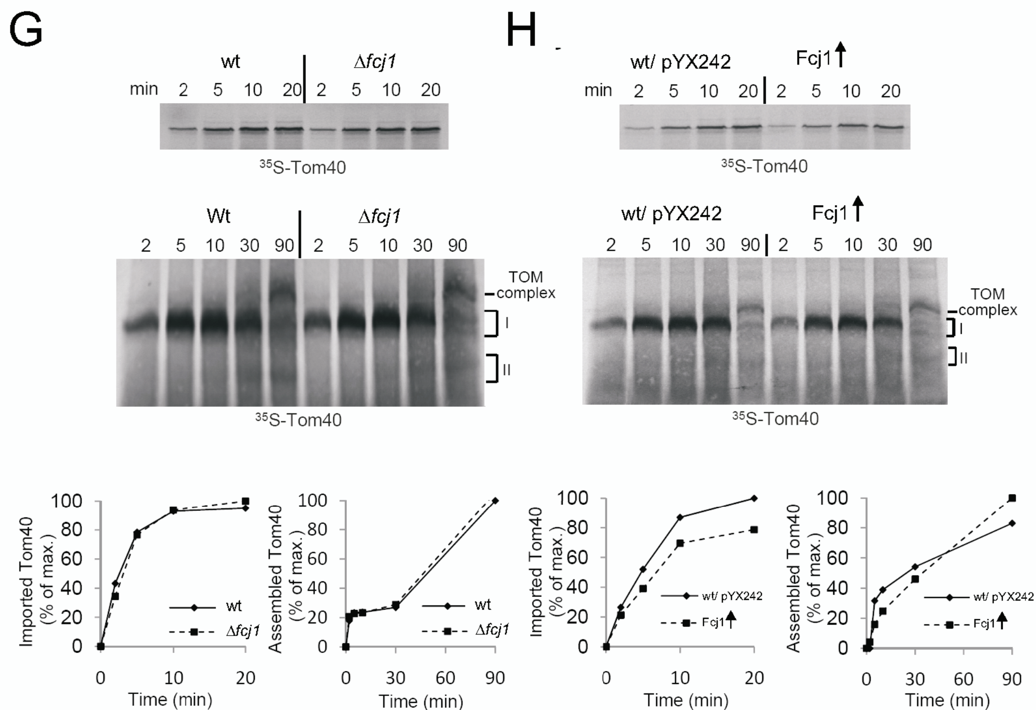


Körner *et al.* 2012 Figure S4

Supplementary Figure S4. **Mitochondrial morphology in cells expressing Fcj1 variants. A,** Strains expressing mitochondrial targeted GFP were grown on non-fermentable selective medium and mitochondria were visualized by fluorescence microscopy. **B,** Quantative analysis of mitochondrial morphology. The frequency of cells exhibiting tubular, swollen or fragmented mitochondrial morphology was determined in 50 to 150 cells per strain.



Körner *et al.* 2012 Figure S5A-F



Supplementary Figure S5. Fcj1 has a minor effect on the biogenesis of beta-barrel proteins. A, The FCJ1 protein complex exists in a subpopulations co-migrating with Tob55. Wild type mitochondria were solubilized in digitonin and native protein complexes were separated on a linear 10-50% glycerol gradient by ultracentrifugation. Fractions were analyzed by SDS-PAGE and immunoblotting. **B,** Downregulation of TOB-complex does not alter steady-state levels of Fcj1**.** Wild type cells and cells expressing Tob38 under the control of the *GAL10* promoter were grown on lactate containing 0.1% glucose for down-regulation of the TOB complex. Equal protein amounts of isolated mitochondria from wild type and *GAL10*-Tob38 strains were subjected to SDS-PAGE and analyzed by immunoblotting with antibodies against the indicated proteins. For each sample increasing amounts (6.25, 12.5, and 25 μg) were loaded as indicated. **C,** Overexpression of Tob55 does not alter steady-state levels of Fcj1**.** Wild type cells and cells expressing Tob55 under the control of the *GAL10* promoter were grown on lactate medium containing 2% galactose for overexpression of Tob55. Equal protein amounts of isolated mitochondria from wild type and *GAL10*-Tob55 strains were subjected to SDS-PAGE and analyzed by immunoblotting with antibodies against the indicated proteins. For each sample increasing amounts (6.25, 12.5, and 25 μg) were loaded as indicated. **D and E,** Deletion of Fcj1 does not affect porin biogenesis. Mitochondria were isolated from *Δfcj1* and their corresponding wild type cells and incubated with radiolabeled precursor of porin for the indicated time periods. **D,** Samples were treated with proteinase K and mitochondria were analyzed by SDS-PAGE and autoradiography (left panel). The amounts of imported porin were quantified (right panel). The amount of imported porin into wild type mitochondria after the longest incubation period was set to 100%. **E,** After import reaction, samples were solubilized in 1% digitonin and

analyzed by BN-PAGE and autoradiography (left panel). Assembled porin complexes are indicated. Bands corresponding to assembled porin were quantified and the amount of assembled protein in wild type mitochondria after the longest incubation period was set to 100% (right panel). **F,** Deletion of Fcj1 does not affect Tob55 import. Mitochondria were isolated from either cells lacking Fcj1 (*Δfcj1*) and from the corresponding wild type (wt) cells. Equal amounts of mitochondria were incubated with radiolabeled precursor of Tob55 for various periods of time. Samples were treated with PK and analyzed by SDS-PAGE and autoradiography (left panel). Two characteristic proteolytic fragments of imported Tob55, with molecular masses of about 30 and 25 kDa are indicated as F´ and F´´, respectively (Habib et al., 2005). The bands corresponding to F´ were quantified (bottom panel). The intensity of the fragment formed upon import of Tob55 into wild type mitochondria for the longest incubation period was set to 100%. **G and H,** Overexpression of Fcj1 but not deletion of Fcj1 changes import and assembly kinetics of Tom40. **G,** Mitochondria were isolated from *Δfcj1* and the corresponding wild type cells and incubated with radiolabeled precursor of Tom40 for various time periods. Upper panels: samples were treated with proteinase K and mitochondria were analyzed by SDS-PAGE and autoradiography. The amounts imported Tom40 were quantified. The amount of Tom40 imported into wild type mitochondria after the longest incubation period was set to 100%. Lower panels: after import samples were solubilized in 1% digitonin and analyzed by BN-PAGE and autoradiography. Assembly intermediates (intermediates I and II) and the assembled TOM complex are indicated. Bands corresponding to the assembled TOM complex were quantified and the amount of assembled TOM complex in wild type mitochondria after the longest incubation period was set to 100%. **H,** Mitochondria isolated from the wild type control strain (wt/pYX242) or from the strain overexpressing Fcj1 (Fcj1↑) were incubated with radiolabeled precursor of Tom40 for various time periods. Further treatment and data analysis were as described in G.

Supplementary Table S1. Strains

Name	Description	Background/ genotype	Plasmids	Source
WT	WT	W303α	-	(Rabl *et al.*, 2009)
Δ*fcj1*	Δ*fcj1*	W303α	-	(Rabl *et al.*, 2009)
WT + pYX242	WT	W303α	pYX242	this study
Δ*fcj1* + pYX242	Δ*fcj1*	W303α	pYX242	this study
Δfcj1 + $Fcj1_{wt}$	$Fcj1_{wt}$	W303α	pYX242- $Fcj1_{wt}$	this study
Δfcj1 + $Fcj1_{His}$	$Fcj1_{His}$	W303α	pYX242- $Fcj1_{His}$	(Rabl *et al.*, 2009)
Δfcj1 + $Fcj1_{Dld}$	$Fcj1_{Dld}$	W303α	pYX242- $Fcj1_{Dld}$	this study
Δfcj1 + $Fcj1_{G52L}$	$Fcj1_{G52L}$	W303α	pYX242- $Fcj1_{G52L}$	this study
Δfcj1 + Cyt*b*$_2$-Fcj1	Cyt*b*$_2$-Fcj1	W303α	pYX242- Cyt*b*$_2$-Fcj1	this study
Δfcj1 + $Fcj1_{\Delta 166\text{-}342His}$	$Fcj1_{\Delta 166\text{-}342His}$	W303α	pYX242- $Fcj1_{\Delta 166\text{-}342His}$	this study
$Fcj1_{1\text{-}472}$	$Fcj1_{1\text{-}472}$	W303α	pYX242- $Fcj1_{1\text{-}472}$	this study
$Fcj1_{1\text{-}472His}$	$Fcj1_{1\text{-}472His}$	W303α	pYX242- $Fcj1_{1\text{-}472His}$	this study
Δ*su e*/ pYX242	Δ*su e*/ pYX242	W303α	pYX242	this study
Δ*su e*/ $Fcj1_{wt}$	Δ*su e*/ $Fcj1_{wt}$	W303α	pYX242- $Fcj1_{wt}$	this study
Δ*su e*/ $Fcj1_{His}$	Δ*su e*/ $Fcj1_{His}$	W303α	pYX242- $Fcj1_{His12}$	this study
Δ*su e*/ $Fcj1_{Dld}$	Δ*su e*/ $Fcj1_{Dld}$	W303	pYX242- $Fcj1_{Dld}$	this study
Δ*sue*/$Fcj1_{G52L}$	Δ*sue*/$Fcj1_{G52L}$	W303α	pYX242- $Fcj1_{G52L}$	this study
Δ*su e*/ $Fcj1_{\Delta 166\text{-}342His}$	Δ*su e*/ $Fcj1_{\Delta 166\text{-}342His}$	W303α	pYX242- $Fcj1_{\Delta 166\text{-}342His}$	this study
Δ*su e*/ $Fcj1_{1\text{-}472}$	Δ*su e*/ $Fcj1_{1\text{-}472}$	W303α	pYX242- $Fcj1_{1\text{-}472}$	this study
WT	WT	YPH499	-	
GAL10-TOB38	GAL10-TOB38	YPH499	-	(Waizenegger *et al.*, 2004)
GAL10-TOB55	GAL10-TOB55	YPH499	-	(Paschen *et al.*, 2003)

Supplementary Table S2. Plasmids

Plasmid	Description	Vector	Source
pYX242	Yeast expression vector with constitutive *TPI* promoter	NA	EMD4 Biosciences, USA
pYX242- $Fcj1_{wt}$	Expression of $Fcj1_{wt}$ in yeast	pYX242	(Rabl *et al.*, 2009)
pYX242- $Fcj1_{His}$	Expression of C-terminally His-tagged Fcj1	pYX242	(Rabl *et al.*, 2009)
pYX242- $Fcj1_{Dld1\text{-}TM}$	Expression of Fcj1 with TMD of Dld1	pYX242	this study
pYX242- $Fcj1_{G52L}$	Expression of Fcj1 with G52L mutation in TMD	pYX242	this study
pYX242- $Fcj1_{Cytb2}$	Expression of Fcj1 without TMD after cleavage	pYX242	this study
pYX242- $Fcj1_{\Delta 166\text{-}342His}$	Expression of Coiled-coil deleted Fcj1	pYX242	this study
pYX242- $Fcj1_{1\text{-}472}$	Expression of C-terminally truncated Fcj1	pYX242	this study
pYX242- $Fcj1_{1\text{-}472His}$	Expression of C-terminally truncated Fcj1 with C-terminal His-tag	pYX242	this study
pGEX-$Fcj1_{473\text{-}540}$	Expression of the C-terminal domain of $Fcj1_{473\text{-}540}$ in *E.coli*	pGEX-6P-1	this study
pVTU100-GFP	Expression of mitochondrial GFP	pVTU100	(Westermann and Neupert, 2000)

Supplementary Table S3 Oligonucleotides

Name	Sequence
Fcj1fo	CCC CCC ATG GCA ATG CTA AGA ACT ACT GCC TCA CG
Fcj1rev	CCC CCT CGA GTC ACA ACG TCC TTA TTT CAC AGT CTT C
Fcj1His12rev	CCC CCT CGA GTC AGT GAT GGT GAT GGT GAT GGT GAT GGT GAT GGT GAT GCA ACG TCC TTA TTT CAC AGT CTT C
Fcj1+ DLDfo	CTA CTC TAT TCG GTT ATT TGT TCG CTT CGC AAA AAA ATG ACA AAT TTG GTG AC
Fcj1+DLDrev	AGC GAA CAA ATA ACC GAA TAG AGT AGC TGA AGA GGC GAT GAC AGA GTA ATT GCG AAA TTT GTG CGA AGC C
Fcj1G52Lfo	CAG CGA CAG CTT TCT ACG CCC TAG GTA TCA TAT ATT CGC AAA AAA ATG
Fcj1G52Lrev	CAT TTT TTT GCG AAT ATA TGA TAC CTA GGG CGT AGA AAG CTG TCG CTG
Fcj1dCCrev	CCC CAA GCT TGA GGT TTG AGT CAT TTA GAC TGT TG
Fcj1dCCfo	CAA CAG TCT AAA TGA CTC AAA CCT CAA GCT TAA TAA CTT ACC CGA TGT GAA TAT CG
Fcj1472rev	CCC CCT CGA GTC AAT TAC CTG TCT TTG TGA ATA AGA AAA GG
Fcj1472Hisrev	CCC CCT CGA GTC AGT GAT GGT GAT GGT GAT GGT GAT GGT GAT GGT GAT GAT TAC CTG TCT TTG TGA ATA AGA AAA GG
Fcj1 N57 fo	CCC CGG ATC CTC GCA AAA AAA TGA CAA ATT TGG TGA C
Fcj1 472 fo	CCC CGG ATC CCC TTC AAA TGC CAC GGA TTT CG
Fcj1 rev(BamHI)	GGG GGG ATC CTC ACA ACG TCC TTA TTT CAC AGT CAA G