Supplementary Materials for

Membrane protein insertion through a mitochondrial β-barrel gate

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This PDF file includes:

Other supplementary material for this manuscript includes the following:

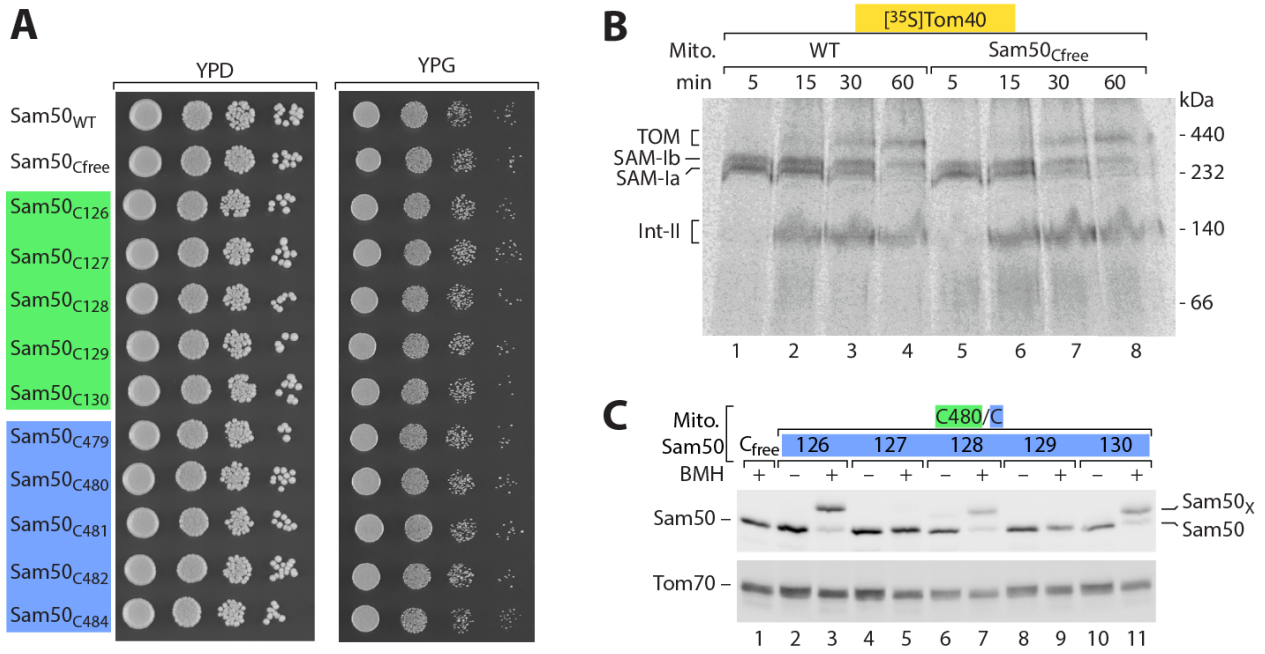

Fig. S1. Characterization of yeast cells expressing Sam50 variants. (**A**) Growth analysis of yeast cells on agar medium containing the fermentable carbon source glucose (YPD) or non-fermentable glycerol (YPG) at 30°C. Cells expressing cysteine-free Sam50 ($Sam50_{Cfree}$) or Sam50 cysteine variants showed a growth behavior comparable to wild-type (WT) cells. (**B**) The import of radiolabeled Tom40 precursor into isolated mitochondria was analyzed by blue native PAGE and autoradiography. The biogenesis steps of [^{35}S]Tom40, including formation of SAM intermediates Ia and Ib, intermediate II (Int-II, dimeric Tom40) and formation of the mature TOM complex, were comparable between WT and $Sam50_{Cfree}$ mitochondria. (**C**) Mitochondria from yeast strains expressing the indicated Sam50 variants were treated with bismaleimidohexane (BMH) and analyzed by SDS-PAGE, Western blotting and immunodecoration with antibodies against Sam50 and Tom70. The lateral Sam50 gate was efficiently crosslinked ($Sam50_X$) with cysteine residues of β strand 1 and β strand 16 pointing into the inside of the Sam50 β-barrel channel.

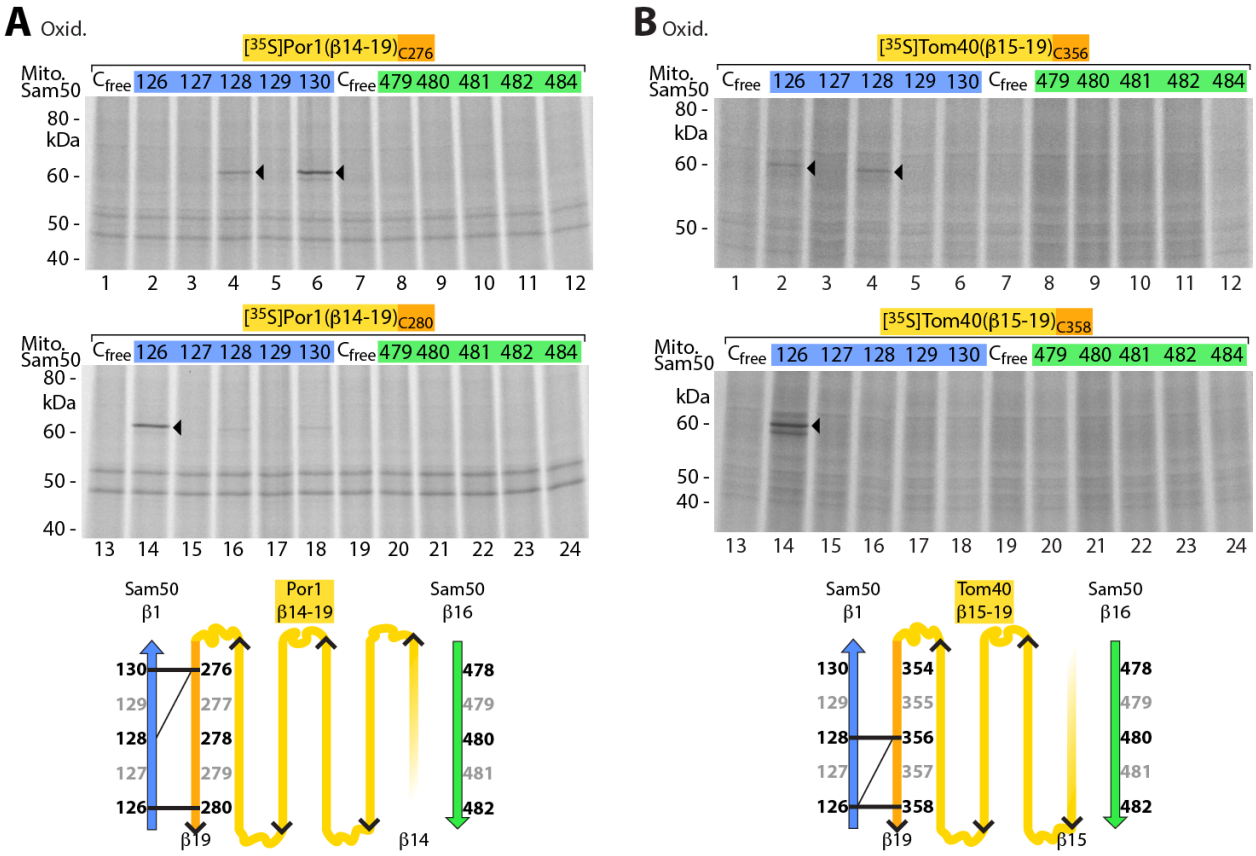

Fig. S2. Interaction of Sam50 with the C-terminal β signal of Por1 and Tom40 precursors. (**A**) [^{35}S]Por1(β14-19)$_{C276}$ and [^{35}S]Por1(β14-19)$_{C280}$ were imported into mitochondria of yeast strains expressing the indicated Sam50 variants, followed by oxidation with 4-DPS. Samples were analyzed by non-reducing SDS-PAGE and autoradiography. Arrowheads, cysteine-specific Sam50-precursor adducts. Schematic model (bottom), disulfide bond formation of Sam50 β strand 1 with the β signal (β19) of the Porin precursor β14-19; thick and thin lines indicate strong and weak formation of Sam50-Por1 adducts, respectively. (**B**) Import of [^{35}S]Tom40$_{C356}$ or [^{35}S]Tom40$_{C358}$ precursor containing β strands 15-19 into isolated mitochondria of yeast strains expressing Sam50 variants was analyzed as in (A). Bottom, model of disulfide bond formation between Sam50 β strand 1 and the β signal (β19) of the Tom40 precursor β15-19.

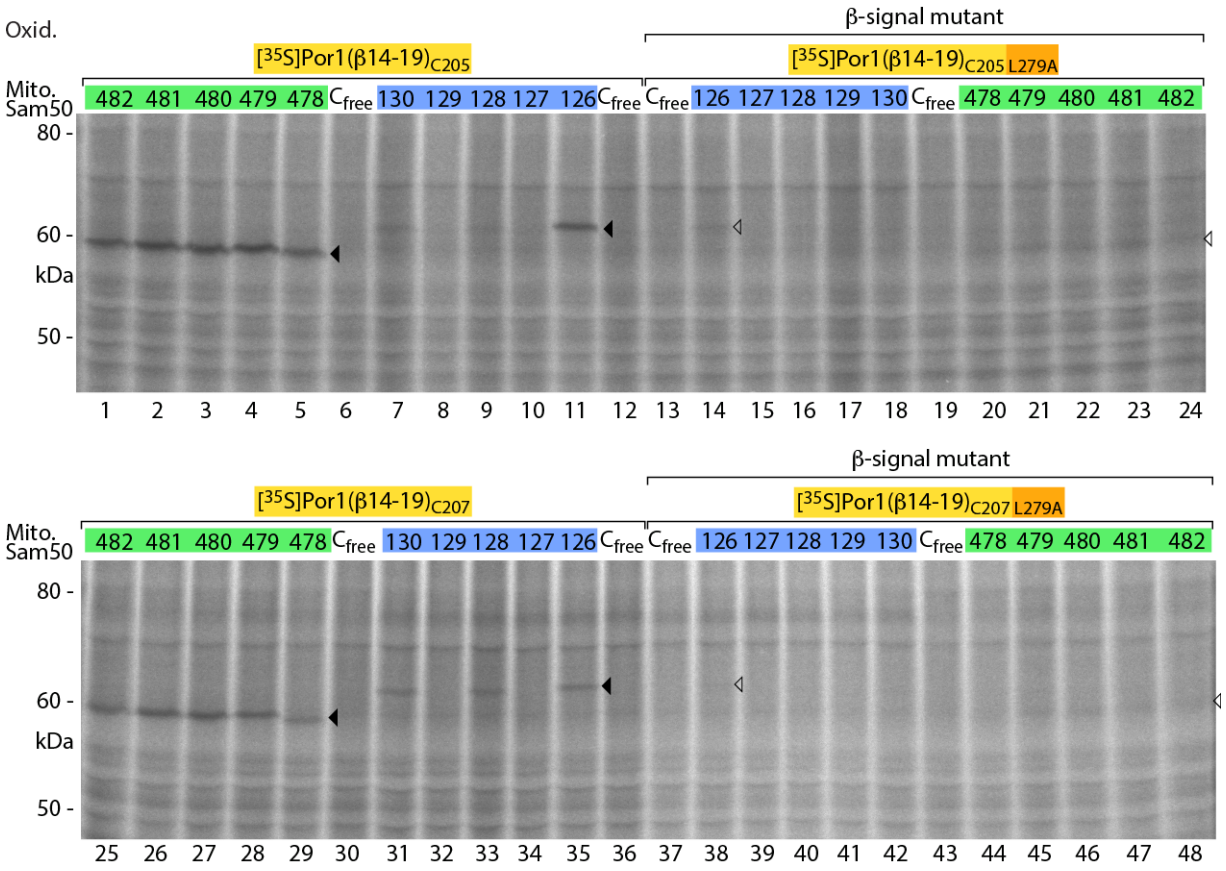

Fig. S3. A mutant β signal impairs the interaction of Por1 constructs with Sam50. Radiolabeled Por1(β14-19)$_{C205}$, Por1(β14-19)$_{C207}$ and the corresponding β-signal mutants (L279A) were incubated for 5 min with isolated Sam50 cysteine variant mitochondria, followed by oxidation with 4-DPS, non-reducing SDS-PAGE and autoradiography. Arrowheads, cysteine-specific Sam50-Por1 precursor adducts.

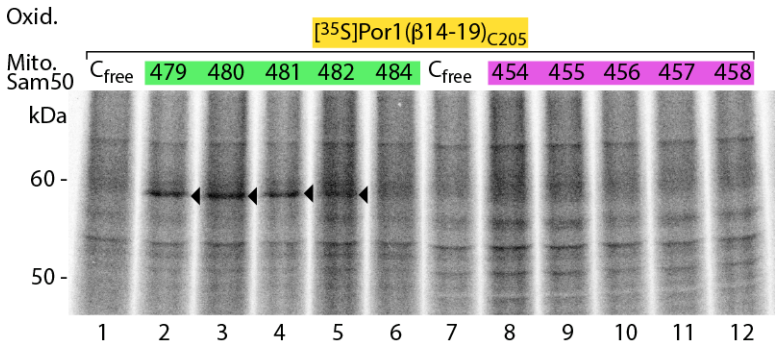

Fig. S4. Interaction of Por1 precursor with β strand 16 of Sam50. [^{35}S]Por1(β14-19)$_{C205}$ was incubated with mitochondria isolated from yeast expressing the indicated Sam50 variants, followed by oxidation with 4-DPS, non-reducing SDS-PAGE and autoradiography. Disulfide formation was observed between the Por1 precursor and Sam50-β16 (arrowheads), but not between Por1 and Sam50-β15.

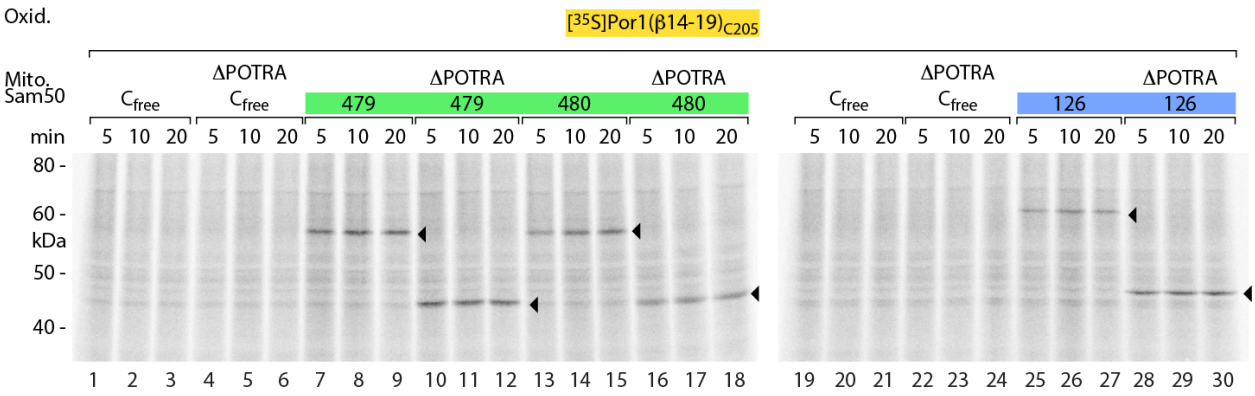

Fig. S5. The POTRA domain of Sam50 is not required for interaction of the Porin precursor with Sam50. [^{35}S]Por1(β14-19)$_{C205}$ was incubated with mitochondria isolated from yeast expressing Sam50 cysteine variants, which lacked the POTRA domain where indicated, for the indicated time. Samples were oxidized using 4-DPS, analyzed by non-reducing SDS-PAGE and autoradiography. Arrowheads, cysteine-specific Sam50-Por1 precursor adducts.

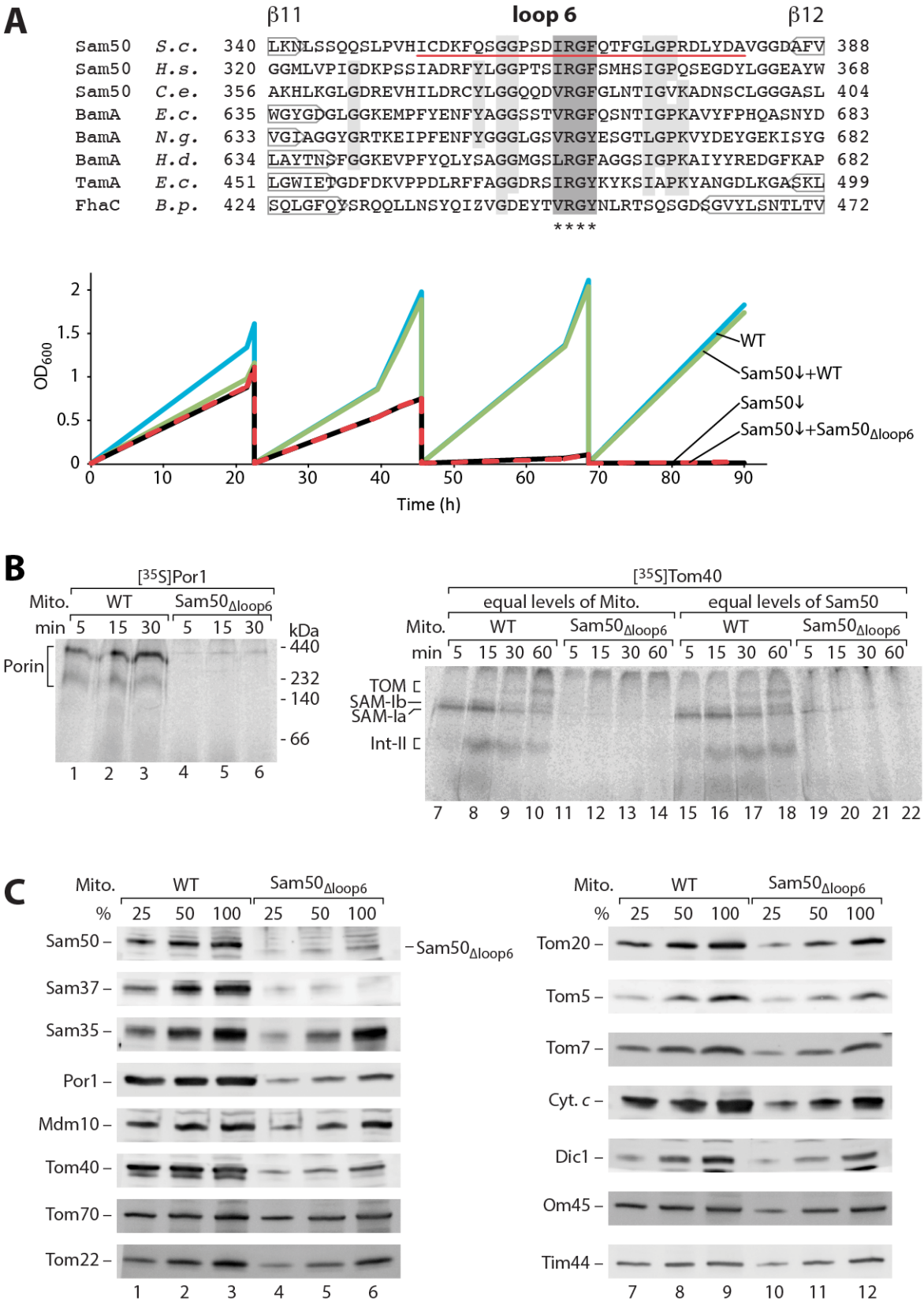
A
β11
loop 6
β12
Sam50 S.c. 340 LKNLSSQQSLPVHICDKFQSGGPSDIRGFQTFGLGPRDLYDAVGGDAFV 388
Sam50 H.s. 320 GGMLVPIGDKPSSIADRFYLGGPTSIRGFSMHSIGPQSEGDYLGGEAYW 368
Sam50 C.e. 356 AKHLKGLGDREVHILDRCYLGGQQDVRGFGLNTIGVKADNSCLGGGASL 404
BamA E.c. 635 WGYGDGLGGKEMPFYENFYAGGSSTVRGFQSNTIGPKAVYFPHQASNYD 683
BamA N.g. 633 VGIAGGYGRTKEIPFENFYGGGLGSVRGYESGTLGPKVYDEYGEKISYG 682
BamA H.d. 634 LAYTNSFGGKEVPFYQLYSAGGMGSLRGFAGGSIGPKAIYYREDGFKAP 682
TamA E.c. 451 LGWIETGDFDKVPPDLRFFAGGDRSIRGYKYKSIAPKYANGDLKGASKL 499
FhaC B.p. 424 SQLGFQYSRQQLLNSYQIZVGDEYTVRGYNLRTSQSGDSGVYLSNTLTV 472
****
OD600
Time (h)
WT
Sam50↓+WT
Sam50↓
Sam50↓+Sam50Δloop6
B
[35S]Por1
Mito.
WT
Sam50Δloop6
min
kDa
Porin
[35S]Tom40
equal levels of Mito.
equal levels of Sam50
TOM
SAM-Ib
SAM-Ia
Int-II
C
Mito.
WT
Sam50Δloop6
%
Sam50 –
Sam37 –
Sam35 –
Por1 –
Mdm10 –
Tom40 –
Tom70 –
Tom22 –
Tom20 –
Tom5 –
Tom7 –
Cyt. c –
Dic1 –
Om45 –
Tim44 –

Fig. S6

Fig. S6. Characterization of Sam50$_{\Delta loop6}$ mitochondria. (**A**) Upper panel, Omp85 proteins were aligned based on their conserved IRGF motif (dark gray). The amino acid residues affected by the deletion of the conserved part of loop 6 in *S.c.* Sam50 are underlined in red (Sam50$_{\Delta loop6}$). *S.c., Saccharomyces cerevisiae*; *H.s.*, *Homo sapiens*; *C.e.*, *Caenorhabditis elegans* (P46576, SAM50-like protein gop-3); *E.c., Escherichia coli*; *N.g., Neisseria* gonorrhoeae; *H.d.*, *Haemophilus ducreyi*; *B.p., Bordetella pertussis*. TamA (*19*); FhaC (*78*). Lower panel, growth of wild-type (WT, blue), Sam50↓ (*SAM50* down-regulated, black), Sam50↓ expressing *SAM50* WT (green) and Sam50↓ expressing Sam50$_{\Delta loop6}$ (red) reveals that Sam50-loop 6 is essential. (**B**) Assembly of [^{35}S]Por1 and [^{35}S]Tom40 analyzed by blue native PAGE is blocked in Sam50$_{\Delta loop6}$ mitochondria. Samples 19-22, the amounts of mitochondria were four-fold increased to compensate for the decreased levels of Sam50 in Sam50$_{\Delta loop6}$ mitochondria. (**C**) Protein levels of WT and Sam50$_{\Delta loop6}$ mitochondria were analyzed by SDS-PAGE and immunodecoration.

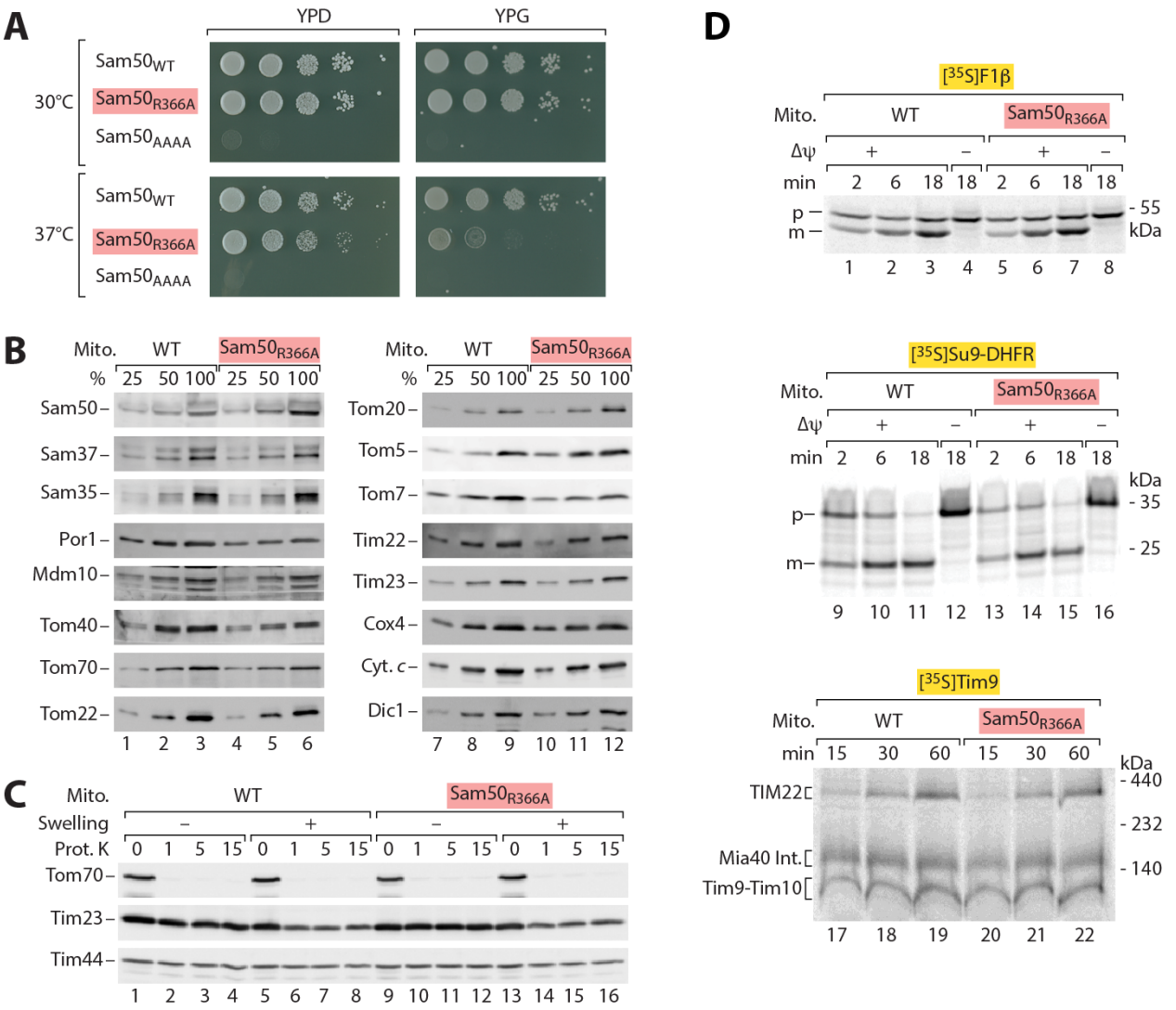

Fig. S7. Characterization of Sam50-loop 6 IRGF motif variants. (**A**) Growth of yeast cells expressing Sam50, $Sam50_{AAAA}$ and $Sam50_{R366A}$ on fermentable (YPD) and non-fermentable (YPG) medium at 30°C and 37°C. Cells expressing Sam50 with four alanine residues instead of the conserved IRGF motif showed a strong growth defect. Cells expressing $Sam50_{R366A}$ showed a temperature sensitive growth behavior on YPG. (**B**) Growth of wild-type (WT) and $Sam50_{R366A}$ cells at permissive temperature (24°C) yielded isolated mitochondria with comparable steady state protein levels of WT and mutant, as analyzed by SDS-PAGE and immunoblotting. (**C**) The outer membranes of both WT and $Sam50_{R366A}$ mitochondria protected internal mitochondrial proteins against proteinase K (Prot. K) under isotonic conditions (- swelling). After disruption of the outer membrane under hypotonic conditions (+ swelling), the intermembrane space exposed protein Tim23 was degraded by the protease as revealed by SDS-PAGE and immunoblotting. The matrix protein Tim44 remained intact under both conditions. Thus, the outer membrane of $Sam50_{R366A}$ mitochondria is intact, excluding an indirect inhibition of β-barrel biogenesis caused by a loss of intermembrane space chaperones (*57, 58*). (**D**) The presequence-carrying matrix precursors [^{35}S]F_1β and [^{35}S]Su9-DHFR, and the intermembrane space-targeted [^{35}S]Tim9 were imported into isolated mitochondria and subjected to SDS-PAGE (lanes 1-16) and blue native PAGE (lanes 17-22), respectively. p, precursor; m, mature; Mia40 Int., Tim9 precursor bound to Mia40.

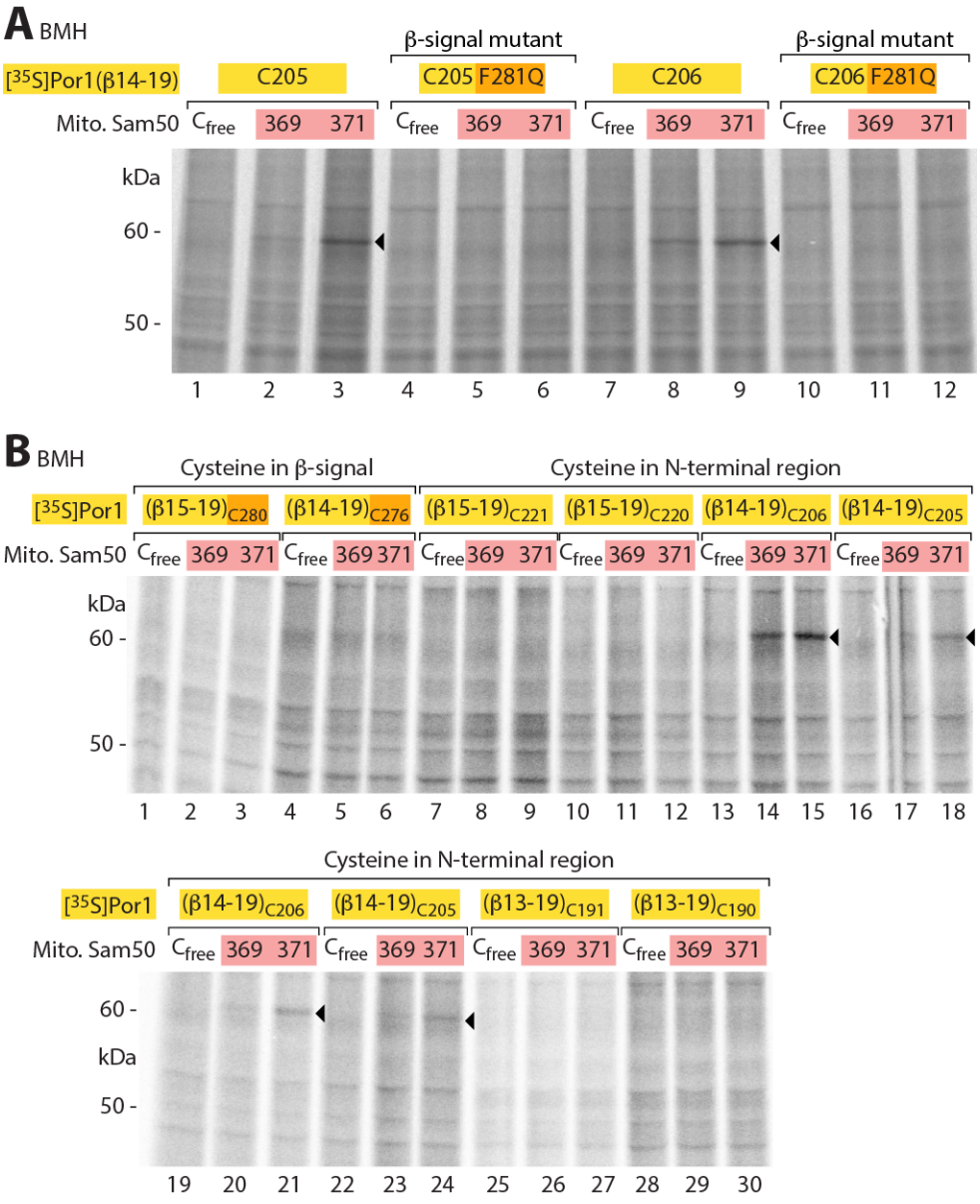

Fig. S8. Interaction of Sam50-loop 6 with different regions of the Porin precursor. (**A**) Radiolabeled Por1(β14-19)$_{C205}$, Por1(β14-19)$_{C206}$ and their corresponding β-signal mutant (F281Q) were incubated for 5 min with isolated Sam50 mutant mitochondria as indicated. Samples were treated with BMH and analyzed by non-reducing SDS-PAGE and autoradiography. (**B**) Radiolabeled Por1 constructs were imported for 5 min into mitochondria as indicated. Samples were treated as in (A). Arrowheads, cysteine-specific Sam50-Por1 precursor adducts.

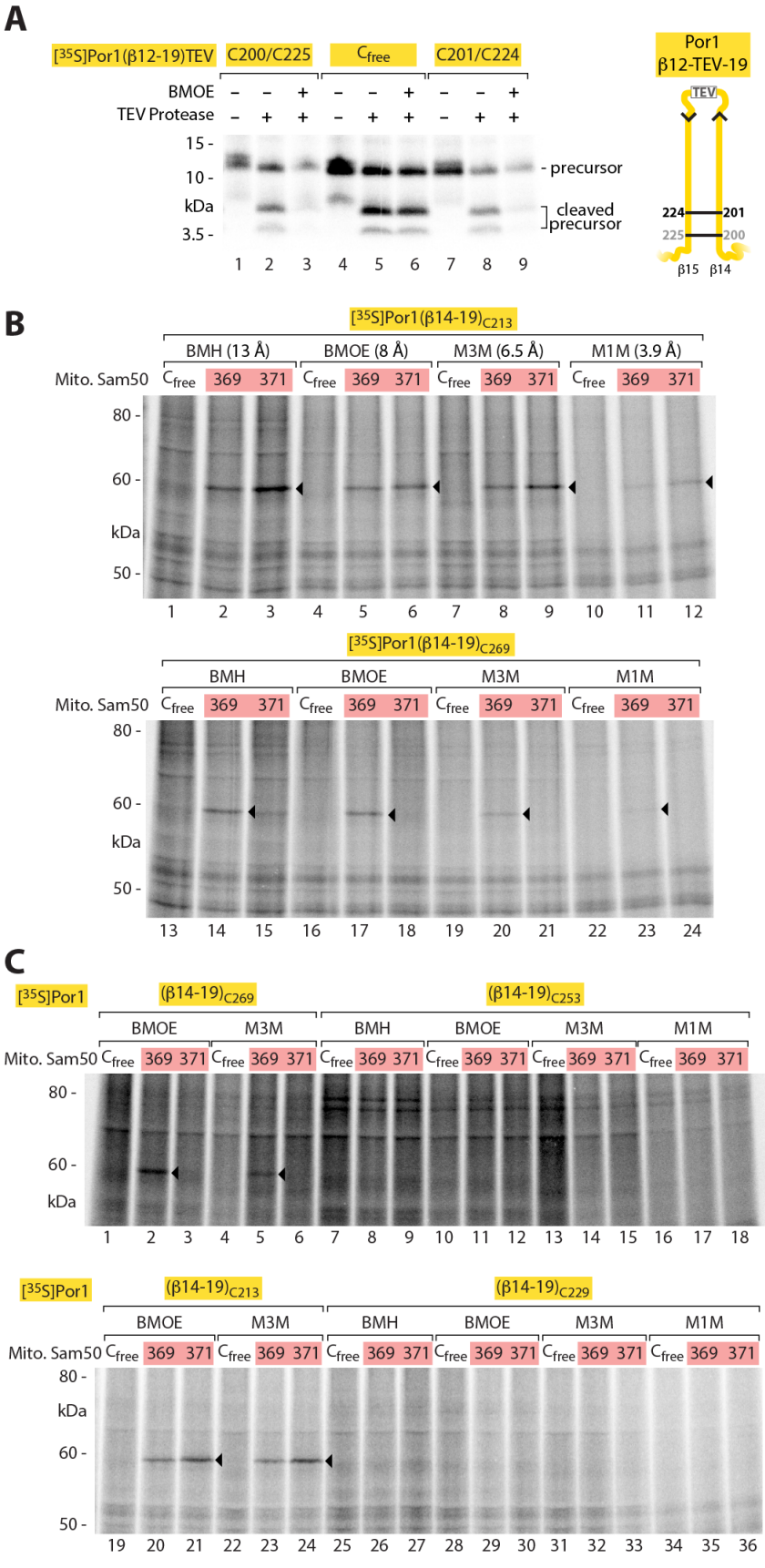
A
[35S]Por1(β12-19)TEV
C200/C225
Cfree
C201/C224
BMOE
TEV Protease
15 -
10 -
kDa
3.5 -
- precursor
cleaved precursor
1 2 3 4 5 6 7 8 9
Por1 β12-TEV-19
TEV
224
201
225
200
β15
β14
B
[35S]Por1(β14-19)C213
BMH (13 Å)
BMOE (8 Å)
M3M (6.5 Å)
M1M (3.9 Å)
Mito. Sam50
Cfree 369 371
80 -
60 -
kDa
50 -
1 2 3 4 5 6 7 8 9 10 11 12
[35S]Por1(β14-19)C269
BMH
BMOE
M3M
M1M
13 14 15 16 17 18 19 20 21 22 23 24
C
[35S]Por1
(β14-19)C269
(β14-19)C253
BMOE
M3M
BMH
M1M
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
[35S]Por1
(β14-19)C213
(β14-19)C229
19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36

Fig. S9

Fig. S9. Characterization of Por1 precursor accumulated at SAM. (**A**) Radiolabeled Por1 constructs containing a TEV protease cleavage site were imported into $Sam50_{Cfree}$ mitochondria for 15 min. Samples were crosslinked using bismaleimidoethane (BMOE). After cleavage by TEV protease, samples were analyzed on a non-reducing Nu-PAGE gel and autoradiography. (Right) Schematic model of the location of the TEV cleavage site and the cysteines used. (**B**) $[^{35}S]Por1(\beta 14\text{-}19)_{C213}$ and $[^{35}S]Por1(\beta 14\text{-}19)_{C269}$ were imported into mitochondria isolated from yeast cells expressing the indicated Sam50 variants for 5 min. Samples were crosslinked using either BMH, BMOE, 1,3-propanediylbismethanethiosulfonat (M3M) and 1,1-methanediylbismethanethiosulfonat (M1M) as indicated. The length of the crosslinkers is stated in parenthesis. Samples were analyzed by non-reducing SDS-PAGE and autoradiography. (**C**) Radiolabeled Por1 constructs were imported into Sam50 mutant mitochondria as indicated. Samples were treated as in (B). Arrowheads, cysteine-specific Sam50-Por1 adducts. The findings of (B) and (C) are summarized in Fig. 7B.

Table S1. List of plasmids used in this study.

Cf and Cfree, cysteine free.

Plasmid name	Expressed protein	Promoter	Vector	Primers for mutagenesis or source	Method	Template DNA or source	Number
pFL39	–	–	pFL39	–	–	*66*	X15
pFL39Sam50	Sam50	*SAM50*	pFL39	–	–	*13*	1699
pFL39Sam50$_{R366A}$	Sam50$_{R366A}$	*SAM50*	pFL39	R366A	Site-directed mutagenesis	pFL39Sam50	2072
pFL39Sam50$_{AAAA}$	Sam50$_{AAAA}$	*SAM50*	pFL39	AAAA	Site-directed mutagenesis	pFL39Sam50	2073
pFL39Sam50$_{\Delta loop6}$	Sam50$_{\Delta 353-381}$	*SAM50*	pFL39	Δloop6	Site-directed mutagenesis	pFL39Sam50	2071
pFL39Sam50$_{Cfree}$	Sam50$_{Cfree}$	*SAM50*	pFL39	C76K; C245V; C252G; C292L; C354A; C430H	Site-directed mutagenesis	pFL39Sam50	2690
pFL39Sam50ΔPOTRA$_{Cfree}$	Sam50ΔPOTRA$_{Cfree}$	*SAM50*	pFL39	*13*	Site-directed mutagenesis	pFL39Sam50Cf	2773
pFL39Sam50ΔPOTRA$_{C126}$	Sam50ΔPOTRA$_{C126}$	*SAM50*	pFL39	K126C	Site-directed mutagenesis	pFL39Sam50 ΔPOTRA$_{Cfree}$	2774
pFL39Sam50ΔPOTRA$_{C479}$	Sam50ΔPOTRA$_{C479}$	*SAM50*	pFL39	L479C	Site-directed mutagenesis	pFL39Sam50 ΔPOTRA$_{Cfree}$	2775
pFL39Sam50ΔPOTRA$_{C480}$	Sam50ΔPOTRA$_{C480}$	*SAM50*	pFL39	G480C	Site-directed mutagenesis	pFL39Sam50 ΔPOTRA$_{Cfree}$	2776
pFL39Sam50Cf$_{C478/C126}$	Sam50Cf$_{C478/C126}$	*SAM50*	pFL39	K126C	Site-directed mutagenesis	pFL39Sam50Cf$_{C478}$	2787
pFL39Sam50Cf$_{C478/C127}$	Sam50Cf$_{C478/C127}$	*SAM50*	pFL39	T127C	Site-directed mutagenesis	pFL39Sam50Cf$_{C478}$	2788
pFL39Sam50Cf$_{C478/C128}$	Sam50Cf$_{C478/C128}$	*SAM50*	pFL39	G128C	Site-directed mutagenesis	pFL39Sam50Cf$_{C478}$	2789
pFL39Sam50Cf$_{C478/C129}$	Sam50Cf$_{C478/C129}$	*SAM50*	pFL39	T129C	Site-directed mutagenesis	pFL39Sam50Cf$_{C478}$	2790
pFL39Sam50Cf$_{C478/C130}$	Sam50Cf$_{C478/C130}$	*SAM50*	pFL39	N130C	Site-directed mutagenesis	pFL39Sam50Cf$_{C478}$	2791
pFL39Sam50Cf$_{C480/C126}$	Sam50Cf$_{C480/C126}$	*SAM50*	pFL39	K126C	Site-directed mutagenesis	pFL39Sam50Cf$_{C480}$	2708
pFL39Sam50Cf$_{C480/C127}$	Sam50Cf$_{C480/C127}$	*SAM50*	pFL39	T127C	Site-directed mutagenesis	pFL39Sam50Cf$_{C480}$	2709
pFL39Sam50Cf$_{C480/C128}$	Sam50Cf$_{C480/C128}$	*SAM50*	pFL39	G128C	Site-directed mutagenesis	pFL39Sam50Cf$_{C480}$	2710
pFL39Sam50Cf$_{C480/C129}$	Sam50Cf$_{C480/C129}$	*SAM50*	pFL39	T129C	Site-directed mutagenesis	pFL39Sam50Cf$_{C480}$	2711
pFL39Sam50Cf$_{C480/C130}$	Sam50Cf$_{C480/C130}$	*SAM50*	pFL39	N130C	Site-directed mutagenesis	pFL39Sam50Cf$_{C480}$	2717
pFL39Sam50Cf$_{C482/C126}$	Sam50Cf$_{C482/C126}$	*SAM50*	pFL39	K126C	Site-directed mutagenesis	pFL39Sam50Cf$_{C482}$	2792
pFL39Sam50Cf$_{C482/C127}$	Sam50Cf$_{C482/C127}$	*SAM50*	pFL39	T127C	Site-directed mutagenesis	pFL39Sam50Cf$_{C482}$	2793
pFL39Sam50Cf$_{C482/C128}$	Sam50Cf$_{C482/C128}$	*SAM50*	pFL39	G128C	Site-directed mutagenesis	pFL39Sam50Cf$_{C482}$	2794

pFL39Sam50Cf$_{C482/C129}$	Sam50Cf$_{C482/C129}$	*SAM50*	pFL39	T129C	Site-directed mutagenesis	pFL39Sam50Cf$_{C482}$	2795
pFL39Sam50Cf$_{C482/C130}$	Sam50Cf$_{C482/C130}$	*SAM50*	pFL39	N130C	Site-directed mutagenesis	pFL39Sam50Cf$_{C482}$	2796
pFL39Sam50Cf$_{C126}$	Sam50Cf$_{C126}$	*SAM50*	pFL39	K126C	Site-directed mutagenesis	pFL39Sam50$_{Cfree}$	2691
pFL39Sam50Cf$_{C127}$	Sam50Cf$_{C127}$	*SAM50*	pFL39	T127C	Site-directed mutagenesis	pFL39Sam50$_{Cfree}$	2692
pFL39Sam50Cf$_{C128}$	Sam50Cf$_{C128}$	*SAM50*	pFL39	G128C	Site-directed mutagenesis	pFL39Sam50$_{Cfree}$	2693
pFL39Sam50Cf$_{C129}$	Sam50Cf$_{C129}$	*SAM50*	pFL39	T129C	Site-directed mutagenesis	pFL39Sam50$_{Cfree}$	2694
pFL39Sam50Cf$_{C130}$	Sam50Cf$_{C130}$	*SAM50*	pFL39	N130C	Site-directed mutagenesis	pFL39Sam50$_{Cfree}$	2695
pFL39Sam50Cf$_{C369}$	Sam50Cf$_{C369}$	*SAM50*	pFL39	Q369C	Site-directed mutagenesis	pFL39Sam50$_{Cfree}$	2718
pFL39Sam50Cf$_{C371}$	Sam50Cf$_{C371}$	*SAM50*	pFL39	F371C	Site-directed mutagenesis	pFL39Sam50$_{Cfree}$	2719
pFL39Sam50Cf$_{C454}$	Sam50Cf$_{C454}$	*SAM50*	pFL39	R454C	Site-directed mutagenesis	pFL39Sam50$_{Cfree}$	2800
pFL39Sam50Cf$_{C455}$	Sam50Cf$_{C455}$	*SAM50*	pFL39	F455C	Site-directed mutagenesis	pFL39Sam50$_{Cfree}$	2797
pFL39Sam50Cf$_{C456}$	Sam50Cf$_{C456}$	*SAM50*	pFL39	E456C	Site-directed mutagenesis	pFL39Sam50$_{Cfree}$	2798
pFL39Sam50Cf$_{C457}$	Sam50Cf$_{C457}$	*SAM50*	pFL39	L457C	Site-directed mutagenesis	pFL39Sam50$_{Cfree}$	2799
pFL39Sam50Cf$_{C458}$	Sam50Cf$_{C458}$	*SAM50*	pFL39	N458C	Site-directed mutagenesis	pFL39Sam50$_{Cfree}$	2801
pFL39Sam50Cf$_{C478}$	Sam50Cf$_{C478}$	*SAM50*	pFL39	G478C	Site-directed mutagenesis	pFL39Sam50$_{Cfree}$	2701
pFL39Sam50Cf$_{C479}$	Sam50Cf$_{C479}$	*SAM50*	pFL39	L479C	Site-directed mutagenesis	pFL39Sam50$_{Cfree}$	2702
pFL39Sam50Cf$_{C480}$	Sam50Cf$_{C480}$	*SAM50*	pFL39	G480C	Site-directed mutagenesis	pFL39Sam50$_{Cfree}$	2703
pFL39Sam50Cf$_{C481}$	Sam50Cf$_{C481}$	*SAM50*	pFL39	L481C	Site-directed mutagenesis	pFL39Sam50$_{Cfree}$	2704
pFL39Sam50Cf$_{C482}$	Sam50Cf$_{C482}$	*SAM50*	pFL39	A482C	Site-directed mutagenesis	pFL39Sam50$_{Cfree}$	2705
pFL39Sam50Cf$_{C484}$	Sam50Cf$_{C484}$	*SAM50*	pFL39	L484C	Site-directed mutagenesis	pFL39Sam50$_{Cfree}$	2707
pFL39Por1	Por1	*POR1*	pFL39	–	–	Wiedemann/Pfanner Labs	1696
pFL39Por1$_{Cfree}$	Por1$_{Cfree}$	*POR1*	pFL39	–	–	*39*	1987
pFL39Por1TEV$_{Cfree}$	Por1TEV$_{Cfree}$	*POR1*	pFL39	TEV	Site-directed mutagenesis	pFL39Por1$_{Cfree}$	2780
pFL39Por1TEV$_{C200/C225}$	Por1TEV$_{C200/C225}$	*POR1*	pFL39	L200C; Y225C	Site-directed mutagenesis	pFL39Por1TEV$_{Cfree}$	2781
pFL39Por1TEV$_{C201/C224}$	Por1TEV$_{C201/C224}$	*POR1*	pFL39	Q201C; R224C	Site-directed mutagenesis	pFL39Por1TEV$_{Cfree}$	2782
pFL39Por1Cf$_{C51}$	Por1Cf$_{C51}$	*POR1*	pFL39	D51C	Site-directed mutagenesis	pFL39Por1$_{Cfree}$	2787
pFL39Por1Cf$_{C220}$	Por1Cf$_{C220}$	*POR1*	pFL39	E220C	Site-directed mutagenesis	pFL39Por1$_{Cfree}$	2685
pFL39Por1Cf$_{C221}$	Por1Cf$_{C221}$	*POR1*	pFL39	F221C	Site-directed mutagenesis	pFL39Por1$_{Cfree}$	2684
pFL39Por1Cf$_{C204}$	Por1Cf$_{C204}$	*POR1*	pFL39	A204C	Site-directed mutagenesis	pFL39Por1$_{Cfree}$	2688
pFL39Por1Cf$_{C205}$	Por1Cf$_{C205}$	*POR1*	pFL39	K205C	Site-directed mutagenesis	pFL39Por1$_{Cfree}$	2687
pFL39Por1Cf$_{C206}$	Por1Cf$_{C206}$	*POR1*	pFL39	A206C	Site-directed mutagenesis	pFL39Por1$_{Cfree}$	2686
pFL39Por1Cf$_{C207}$	Por1Cf$_{C207}$	*POR1*	pFL39	T207C	Site-directed mutagenesis	pFL39Por1$_{Cfree}$	2802

pFL39Por1Cf$_{C159}$	Por1Cf$_{C159}$	*POR1*	pFL39	A159C	Site-directed mutagenesis	pFL39Por1$_{Cfree}$	2779
pFL39Por1Cf$_{C213}$	Por1Cf$_{C213}$	*POR1*	pFL39	P213C	Site-directed mutagenesis	pFL39Por1$_{Cfree}$	2783
pFL39Por1Cf$_{C229}$	Por1Cf$_{C229}$	*POR1*	pFL39	A229C	Site-directed mutagenesis	pFL39Por1$_{Cfree}$	2786
pFL39Por1Cf$_{C253}$	Por1Cf$_{C253}$	*POR1*	pFL39	P253C	Site-directed mutagenesis	pFL39Por1$_{Cfree}$	2785
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pFL39Por1Cf$_{C278}$	Por1Cf$_{C278}$	*POR1*	pFL39	S278C	Site-directed mutagenesis	pFL39Por1$_{Cfree}$	2675
pFL39Por1Cf$_{C280}$	Por1Cf$_{C280}$	*POR1*	pFL39	S280C	Site-directed mutagenesis	pFL39Por1$_{Cfree}$	2673
pFL39Tom40$_{Cfree}$	Tom40$_{Cfree}$	*TOM40*	pFL39	–	–	*39*	2104
pFL39Tom40Cf$_{C356}$	Tom40Cf$_{C356}$	*TOM40*	pFL39	G356C	Site-directed mutagenesis	pFL39Tom40$_{Cfree}$	2720
pFL39Tom40Cf$_{C87/C358}$	Tom40Cf$_{C87/C359}$	*TOM40*	pFL39	–	–	*39*	2010
pGEM-4Z-Tom40	Tom40	*SP6*	pGEM-4Z	–	–	Wiedemann/Pfanner Labs	1495
pGEM-4Z-Por1	Por1	*SP6*	pGEM-4Z	–	–	Wiedemann/Pfanner Labs	P14
pSP64-F1β	F1β	*SP6*	pSP64	–	–	Wiedemann/Pfanner Labs	F01
pGEM-4Z-Mdm10	Mdm10	*SP6*	pGEM-4Z	–	–	Wiedemann/Pfanner Labs	2148
pGEM-4Z-Su9-DHFR	Su9-DHFR	*SP6*	pGEM-4Z	–	–	Wiedemann/Pfanner Labs	S02
pGEM-4Z-Tim9	Tim9	*SP6*	pGEM-4Z	–	–	Wiedemann/Pfanner Labs	JR59
pGEX-4T-1-GST-β-signalPor1	GST-β-signal$_{Por1}$	*tac*	pGEX-4T-1	–	–	*13*	1719
pGEX-4T-1-GST-β-signalPor1F281Q	GST-β-signal$_{Por1F281Q}$	*tac*	pGEX-4T-1	–	–	*13*	1720
pFA6AHis3MX6-pGal1	–	–	pFA6	–	–	*68*	1439

Table S2. List of primers.

For cloning (codons causing amino acid exchange are underlined).

Mutation		Primer forward	Primer reverse
Gene	**Position**		
SAM50	R366A	GGTGGGCCCAGCGACATTGCGGGGTTTCAA	TTGAAACCCCGCAATGTCGCTGGGCCCACC
	AAAA	CAGAGTGGTGGGCCCAGCGACGCTGCGGCGGCTCAAACATTTGGGTTAGGCCC	GGGCCTAACCCAAATGTTTGAGCCGCCGCAGCGTCGCTGGGCCCACCACTCTG
	Δloop6	CTCACAACAATCCTTGCCTGTAGGTGGTGATGCTTTTG	CAAAAGCATCACCACCTACAGGCAAGGATTGTTGTGAG
	C76K	TAAGAGATTGAAGCAACACCACATTG	TGTGGTGTTGCTTCAATCTCTTATTTAAC
	C245V	TTTAGAGACCGTCTGGAGATCC	GGATCTCCAGACGGTCTCTAAC
	C252G	CACTAAGATAGGTTCACAAGG	CCTTGTGAACCTATCTTAGTG
	C292L	TCATATTATGCTCCCTACTAAG	ACCCTTAGTAGGGAGCATAATATGATC
	C354A	TGTACATATAGCTGATAAGTTTC	TGAAACTTATCAGCTATATGTAC
	C430H	GTTGGGTAATCACATAGGACA	CTGTCCTATGTGATTACCCAA
	K126C	GTTTACAGCGTGCACAGGGACAAATTTTGGG	GTCCCTGTGCACGCTGTAAACGTTTTAGGAG
	T127C	CAGCGAAGTGCGGGACAAATTTTGGGAACGATAATG	CCAAAATTTGTCCGCACTTCGCTGTAAACGTTTTAGG
	G128C	GAAGACATGCACAAATTTTGGGAACGATAATG	CCAAAATTTGTGCATGTCTTCGCTGTAAACG
	T129C	GACAGGGTGCAATTTTGGGAACGATAATGATGC	CCAAAATTGCACCCTGTCTTCGCTGTAAACG
	N130C	GGACATGTTTTGGGAACGATAATGATGC	CCAAAACATGTCCCTGTCTTCGCTG
	Q369C	GGGTTTTGTACATTTGGGTTAGG	CAAATGTACAAAACCCCCGAATG
	F371C	CAAACATGTGGGTTAGGCCC	CTAACCCACATGTTTGAAACCC
	R454C	CACCCAATGGCATGTTTCGAGCTAAACTTTACTTTGC	GTTTAGCTCGAAACATGCCATTGGGTGCCTTAG
	F455C	CAATGGCAAGATGCGAGCTAAACTTTAC	GTTTAGCTCGCATCTTGCCATTGGGTG
	E456C	GCAAGATTCTGTCTAAACTTTACTTTGCC	GTAAAGTTTAGACAGAATCTTGCCATTGG
	L457C	GCAAGATTCGAGTGTAACTTTACTTTGCC	GTAAAGTTACACTCGAATCTTGCCATTG

Mutation		Primer forward	Primer reverse
Gene	Position		
SAM50	N458C	GATTCGAGCTATGCTTTACTTTGCCTATTACCGCTC	CAAAGTAAAGCATAGCTCGAATCTTGCCATTGGG
	G478C	CCAGTTTTGTCTTGGTCTGGCATTT	GACCAAGACAAAACTGGAATCCTTTTC
	L479C	CCAGTTTGGTTGTGGTCTGGCATTTTTATAAG	CCAGACCACAACCAAACTGGAATCCTTTTC
	G480C	GGTCTTTGTCTGGCATTTTTATAAGAATAC	GCCAGACAAAGACCAAACTGGAATCC
	L481C	CTTGGTTGCGCATTTTTATAAGAATACATTC	CTTATAAAAATGCGCAACCAAGACCAAACTG
	A482C	GGTCTGTGCTTTTTATAAGAATACATTCC	CTTATAAAAAGCACAGACCAAGACCAAACTG
	L484C	GGCATTTTGTTAAGAATACATTCCTTTTAATG	GTATTCTTAACAAAATGCCAGACCAAGAC
POR1	TEV	GAACTCCAAACTACCTGAAAATTTGTATTTTCAAGGTAACTCCAATGTCAACATCGAATTCGCCAC	GTTGACATTGGAGTTACCTTGAAAATACAAATTTTCAGGTAGTTTGGAGTTCATTGTAGCCTTAG
	D51C	CAGCCTGTCAAATGCGGTCCACTGTCTACTAAC	CAGTGGACCGCATTTGACAGGCTGTTTAGCCTTC
	G203C	CTTTTTACAGGTCTGTGCTAAGGCTACAATGAAC	GTAGCCTTAGCACAGACCTGTAAAAAGGCGTTG
	A204C	CAGGTCGGTTGTAAGGCTACAATGAACTCC	CATTGTAGCCTTACAACCGACCTGTAAAAAGG
	K205C	GGTCGGTGCTTGTGCTACAATGAACTCCAAAC	GTTCATTGTAGCACAAGCACCGACCTGTAAAAAGG
	A206C	GGTGCTAAGTGTACAATGAACTCCAAACTACC	GGAGTTCATTGTACACTTAGCACCGACCTG
	T207C	GTCGGTGCTAAGGCTTGTATGAACTCCAAACTACCTAAC	GTTTGGAGTTCATACAAGCCTTAGCACCGACCTG
	A159C	CGATATCAGCTGCGGTTCCATTTCTCGTTATGC	GAAATGGAACCGCAGCTGATATCGTAACCAAACTC
	L200C	CAACGCCTTTTGTCAGGTCGGTGCTAAGG	CACCGACCTGACAAAAGGCGTTGACGTTTTG
	Q201C	GCCTTTTTATGTGTCGGTGCTAAGGCTACAATG	GCACCGACACATAAAAAGGCGTTGACGTTTTG
	P213C	CTCCAAACTATGTAACTCCAATGTCAACATCG	CATTGGAGTTACATAGTTTGGAGTTCATTGTAGC
	E220C	GTCAACATCTGTTTCGCCACTAGATATTTGCC	CTAGTGGCGAAACAGATGTTGACATTGGAG
	F221C	CATCGAATGCGCCACTAGATATTTGCCTGATG	CTAGTGGCGCATTCGATGTTGACATTGGAG
	R224C	ATTCGCCACTTGTTATTTGCCTGATGCATCTTC	CAGGCAAATAACAAGTGGCGAATTCGATGTTG
	Y225C	CCACTAGATGTTTGCCTGATGCATCTTCC	CAGGCAAACATCTAGTGGCGAATTCGATG

Mutation		Primer forward	Primer reverse
Gene	**Position**		
POR1	A229C	TATTTGCCTGATTGCTCTTCC CAAGTTAAGG	CTTGGGAAGAGCAATCAGGC AAATATCTAG
	V253C	ATTGTTAAGATGTGGCGTCA CTCTGGGTG	GAGTGACGCCACATCTTAAC AATTGCTTG
	S269C	CTTTGAAGTTGTGTGAACCT GTTCACAAGCTAG	GAACAGGTTCACACAACTTC AAAGCATCGAAAG
	G276C	CACAAGCTATGTTGGTCTTT GTCCTTCGAC	CAAAGACCAACATAGCTTGT GAACAGGTTCAG
	W277C	CTAGGTTGTTCTTTGTCCTTC GACGCTTG	GGACAAAGAACAACCTAGCT TGTGAACAGG
	S278C	GCTAGGTTGGTGTTTGTCCT TCGACGCTTG	CGAAGGACAAACACCAACCT AGCTTGTGAAC
	S280C	GTCTTTGTGCTTCGACGCTT GAACGTATATATC	CGTCGAAGCACAAAGACCAA CCTAGCTTG
TOM40	G356C	GATTGGTTTCTGTCTACAAT TTGAAACTGCTGG	CAAATTGTAGACAGAAACCA ATCTTGGTATCG
HIS3-Gal1$_{pr}$	R2	–	GAAAATTGGCATCGGGCTGT CTAAGGAAATCTCATTATCA ACACCAGATGATGAGGTCAT TTTGAGATCCGGGTTTT
	F4	GGATGCGTTTTACGTGGCAA AAGTTTTGATGCCAAATAGA CAAAAGTAGCTCAATTCAAC GAATTCGAGCTCGTTTAAAC	–

For in vitro transcription.

Name	Sequence	Primer range (full amino acids)	Gene	Cys
WG-Por1-rev (only Fig. 1C)	TGATGATGAGAACCCCCCCC-TTA-AGCGTCGAAGGACAAAGAC	278-283	*POR1*	-
WG-Por1-b15(C210)-fw (only Fig. 1C)	CTTTAAGAAGGAGATATACC-ATG-*TGC*AAACTACCTAACTCCAATG	210-216	*POR1*	210
rWG-Por1-rev	TGATGATGAGAACCCCCCCC-TCA-CATCATCAT-AGCGTCGAAGGACAAAGACCAAC	277-283	*POR1*	-
rWG-Por1-b15-fw	CTTTAAGAAGGAGATATACC-ATG-AAACTACCTAACTCCAATGTC	211-216	*POR1*	-
rWG-Por1-b14-fw	CTTTAAGAAGGAGATATACC-ATG-ATGATG-AACGTCAACGCCTTTTTACAG	195-201	*POR1*	-
rWG-Por1-b13-fw	CTTTAAGAAGGAGATATACC-ATG-ATGATG-AACGAGCAAATAACTACCGTTG	184-190	*POR1*	-
rWG-Por1-b13(C190)-fw	CTTTAAGAAGGAGATATACC-ATG-ATGATG-AACGAGCAAATAACTACC*TGT*GACTTCTTCC	184-194	*POR1*	190
rWG-Por1-b13(C191)-fw	CTTTAAGAAGGAGATATACC-ATG-ATGATG-AACGAGCAAATAACTACCGTT*TGC*TTCTTCC	184-194	*POR1*	191
rWG-Por1-b12-fw	CTTTAAGAAGGAGATATACC-ATG-ATGATG-GCCAAAGACTACTCCTTGG	173-178	*POR1*	-
rWG-Por1-b12(C179)-fw	CTTTAAGAAGGAGATATACC-ATG-ATGATG-GCCAAAGACTACTCCTTG*TGC*GCTACATTGAAC	173-183	*POR1*	179
rWG-Por1-b12(C180)-fw	CTTTAAGAAGGAGATATACC-ATG-ATGATG-GCCAAAGACTACTCCTTGGGC*TGT*ACATTGAAC	173-183	*POR1*	180

Name	Sequence	Primer range (full amino acids)	Gene	Cys
rWG-Por1-b11-fw	CTTTAAGAAGGAGATATACC-ATG-ATGATG-ATCAGCGCCGGTTCCATTTC	157-162	*POR1*	-
rWG-Por1-b10-fw	CTTTAAGAAGGAGATATACC-ATG-ATGATG-GAAGGTATTGTTGGTGGCGCAGAG	145-152	*POR1*	-
rWG-Por1-b9-fw	CTTTAAGAAGGAGATATACC-ATG-ATGATG-TCACCAACATTTGTTGGTGACTTAAC	133-139	*POR1*	-
rWG-Por1-b8-fw	CTTTAAGAAGGAGATATACC-ATG-ATGATG-CAACCTTTCTTCACCGCAAG	118-123	*POR1*	-
rWG-Por1-b7-fw	CTTTAAGAAGGAGATATACC-ATG-ATGATG-ACTCCAGGCGTCGCCAAG	103-108	*POR1*	-
rWG-Por1-b6-fw	CTTTAAGAAGGAGATATACC-ATG-ATGATG-AACTTGACCCCTGGTCTAAAG	89-95	*POR1*	-
rWG-Por1-b5-fw	CTTTAAGAAGGAGATATACC-ATG-ATGATG-AACACAAACAACTTGCAAACCAAATTAGAGTTTG	77-87	*POR1*	-
rWG-Por1-b4-fw	CTTTAAGAAGGAGATATACC-ATG-ATGATG-GACAAGCAAACCGGCTTGG	63-69	*POR1*	-
rWG-Por1-b3-fw	CTTTAAGAAGGAGATATACC-ATG-ATGATG-GGTCCACTGTCTACTAACG	52-57	*POR1*	-
rWG-Por1-b2-fw	CTTTAAGAAGGAGATATACC-ATG-ATGATG-ACAACCGCCAATGGCATTAAG	34-40	*POR1*	-
rWG-Por1-full-length-fw	CTTTAAGAAGGAGATATACC-ATG-ATGATG-TCTCCTCCAGTTTACAGCGATATC	1-9	*POR1*	-
rWG-Por1-b1(C280)-rev	TGATGATGAGAACCCCCCCC-TTA-CATCATCAT-AGCGTCGAA*GCA*CAAAGAC	277-283	*POR1*	280
rWG-Por1-b1(C278)-rev	TGATGATGAGAACCCCCCCC-TTA-CATCATCAT-AGCGTCGAAGGACAA*ACA*CCAAC	277-283	*POR1*	278
rWG-Por1-b1(C277)-rev	TGATGATGAGAACCCCCCCC-TTA-CATCATCAT-AGCGTCGAAGGACAAAGA*ACA*AC	277-283	*POR1*	277
rWG-Por1-F281Q-rev	TGATGATGAGAACCCCCCCC-TCA-CATCATCAT-AGCGTCGAA*GAA*CAAAGACCAAC	277-283	*POR1*	-
rWG-Por1-L279A-rev	TGATGATGAGAACCCCCCCC-TTA-CATCATCAT-AGCGTCGAAGGA*CGC*AGACCAACC	276-283	*POR1*	-
rWG-Por1-(C276)L279A-rev	TGATGATGAGAACCCCCCCC-TTA-CATCATCAT-AGCGTCGAAGGA*CGC*AGACCA*ACA*	276-283	*POR1*	276
rWG-Por1-(C280)L279A-rev	TGATGATGAGAACCCCCCCC-TTA-CATCATCAT-AGCGTCGAA*GCACGC*AGACCAACC	277-283	*POR1*	280
rWG-Tom40-rev	TGATGATGAGAACCCCCCCC-TTA-CATCATCAT-CAATTGAGGAAGAGCTTGCAATGG	380-378	*TOM40*	-
rWG-Tom40-b15-fw	CTTTAAGAAGGAGATATACC-ATG-ATGATG-CAAGCTGGTATGGTTCCTATTACTG	284-276	*TOM40*	-

Table S3. List of *S. cerevisiae* strains used in this study.

pr, promoter.

Strain name	Strain No.	Genotype	Parental strain	Method used	Source
*sam50*Δ (shuffling strain)	2636	*MAT*a *ura3-52 lys2-801_amber ade2-101_ochre trp1-Δ63 his3-Δ200 leu2-Δ1 sam50::ade2* [Yep352-MET25pr-Sam50-CYC1t]	YPH499	–	*7*
Wild-type (WT) for *sam50* mutants	2630	*MAT*a *ura3-52 lys2-801_amber ade2-101_ochre trp1-Δ63 his3-Δ200 leu2-Δ1 sam50::ade2* [pFL39-Sam50]	*sam50*Δ	Plasmid shuffling	*13*
$Sam50_{R366A}$	3986	*MAT*a *ura3-52 lys2-801_amber ade2-101_ochre trp1-Δ63 his3-Δ200 leu2-Δ1 sam50::ade2* [pFL39-$Sam50_{R366A}$]	*sam50*Δ	Plasmid shuffling	This study
$Sam50_{AAAA}$	3987	*MAT*a *ura3-52 lys2-801_amber ade2-101_ochre trp1-Δ63 his3-Δ200 leu2-Δ1 sam50::ade2* [pFL39-$Sam50_{AAAA}$]	*sam50*Δ	Plasmid shuffling	This study
$Sam50_{Cfree}$	4867	*MAT*a *ura3-52 lys2-801_amber ade2-101_ochre trp1-Δ63 his3-Δ200 leu2-Δ1 sam50::ade2* [pFL39-$Sam50_{Cfree}$]	*sam50*Δ	Plasmid shuffling	This study
Sam50ΔPOTRA$_{Cfree}$	5056	*MAT*a *ura3-52 lys2-801_amber ade2-101_ochre trp1-Δ63 his3-Δ200 leu2-Δ1 sam50::ade2* [pFL39-Sam50ΔPOTRA$_{Cfree}$]	*sam50*Δ	Plasmid shuffling	This study
Sam50ΔPOTRA$_{C126}$	5057	*MAT*a *ura3-52 lys2-801_amber ade2-101_ochre trp1-Δ63 his3-Δ200 leu2-Δ1 sam50::ade2* [pFL39-Sam50ΔPOTRA$_{C126}$]	*sam50*Δ	Plasmid shuffling	This study
Sam50ΔPOTRA$_{C479}$	5058	*MAT*a *ura3-52 lys2-801_amber ade2-101_ochre trp1-Δ63 his3-Δ200 leu2-Δ1 sam50::ade2* [pFL39-Sam50ΔPOTRA$_{C479}$]	*sam50*Δ	Plasmid shuffling	This study
Sam50ΔPOTRA$_{C480}$	5059	*MAT*a *ura3-52 lys2-801_amber ade2-101_ochre trp1-Δ63 his3-Δ200 leu2-Δ1 sam50::ade2* [pFL39-Sam50ΔPOTRA$_{C480}$]	*sam50*Δ	Plasmid shuffling	This study
$Sam50_{C126}$	4868	*MAT*a *ura3-52 lys2-801_amber ade2-101_ochre trp1-Δ63 his3-Δ200 leu2-Δ1 sam50::ade2* [pFL39-Sam50Cf$_{C126}$]	*sam50*Δ	Plasmid shuffling	This study
$Sam50_{C127}$	4869	*MAT*a *ura3-52 lys2-801_amber ade2-101_ochre trp1-Δ63 his3-Δ200 leu2-Δ1 sam50::ade2* [pFL39-Sam50Cf$_{C127}$]	*sam50*Δ	Plasmid shuffling	This study

$Sam50_{C128}$	4870	*MAT*a *ura3-52 lys2-801_amber ade2-101_ochre trp1-Δ63 his3-Δ200 leu2-Δ1 sam50::ade2* [pFL39-$Sam50Cf_{C128}$]	*sam50*Δ	Plasmid shuffling	This study
$Sam50_{C129}$	4871	*MAT*a *ura3-52 lys2-801_amber ade2-101_ochre trp1-Δ63 his3-Δ200 leu2-Δ1 sam50::ade2* [pFL39-$Sam50Cf_{C129}$]	*sam50*Δ	Plasmid shuffling	This study
$Sam50_{C130}$	4872	*MAT*a *ura3-52 lys2-801_amber ade2-101_ochre trp1-Δ63 his3-Δ200 leu2-Δ1 sam50::ade2* [pFL39-$Sam50Cf_{C130}$]	*sam50*Δ	Plasmid shuffling	This study
$Sam50_{C369}$	4908	*MAT*a *ura3-52 lys2-801_amber ade2-101_ochre trp1-Δ63 his3-Δ200 leu2-Δ1 sam50::ade2* [pFL39-$Sam50Cf_{C369}$]	*sam50*Δ	Plasmid shuffling	This study
$Sam50_{C371}$	4909	*MAT*a *ura3-52 lys2-801_amber ade2-101_ochre trp1-Δ63 his3-Δ200 leu2-Δ1 sam50::ade2* [pFL39-$Sam50Cf_{C371}$]	*sam50*Δ	Plasmid shuffling	This study
$Sam50_{C454}$	5074	*MAT*a *ura3-52 lys2-801_amber ade2-101_ochre trp1-Δ63 his3-Δ200 leu2-Δ1 sam50::ade2* [pFL39-$Sam50Cf_{C454}$]	*sam50*Δ	Plasmid shuffling	This study
$Sam50_{C455}$	5070	*MAT*a *ura3-52 lys2-801_amber ade2-101_ochre trp1-Δ63 his3-Δ200 leu2-Δ1 sam50::ade2* [pFL39-$Sam50Cf_{C455}$]	*sam50*Δ	Plasmid shuffling	This study
$Sam50_{C456}$	5071	*MAT*a *ura3-52 lys2-801_amber ade2-101_ochre trp1-Δ63 his3-Δ200 leu2-Δ1 sam50::ade2* [pFL39-$Sam50Cf_{C456}$]	*sam50*Δ	Plasmid shuffling	This study
$Sam50_{C457}$	5072	*MAT*a *ura3-52 lys2-801_amber ade2-101_ochre trp1-Δ63 his3-Δ200 leu2-Δ1 sam50::ade2* [pFL39-$Sam50Cf_{C457}$]	*sam50*Δ	Plasmid shuffling	This study
$Sam50_{C458}$	5073	*MAT*a *ura3-52 lys2-801_amber ade2-101_ochre trp1-Δ63 his3-Δ200 leu2-Δ1 sam50::ade2* [pFL39-$Sam50Cf_{C458}$]	*sam50*Δ	Plasmid shuffling	This study
$Sam50_{C478}$	4873	*MAT*a *ura3-52 lys2-801_amber ade2-101_ochre trp1-Δ63 his3-Δ200 leu2-Δ1 sam50::ade2* [pFL39-$Sam50Cf_{C478}$]	*sam50*Δ	Plasmid shuffling	This study
$Sam50_{C479}$	4874	*MAT*a *ura3-52 lys2-801_amber ade2-101_ochre trp1-Δ63 his3-Δ200 leu2-Δ1 sam50::ade2* [pFL39-$Sam50Cf_{C479}$]	*sam50*Δ	Plasmid shuffling	This study
$Sam50_{C480}$	4875	*MAT*a *ura3-52 lys2-801_amber ade2-101_ochre trp1-Δ63 his3-Δ200 leu2-Δ1 sam50::ade2* [pFL39-$Sam50Cf_{C480}$]	*sam50*Δ	Plasmid shuffling	This study

$Sam50_{C481}$	4876	*MAT*a *ura3-52 lys2-801_amber ade2-101_ochre trp1-Δ63 his3-Δ200 leu2-Δ1 sam50::ade2* [pFL39-$Sam50Cf_{C481}$]	*sam50*Δ	Plasmid shuffling	This study
$Sam50_{C482}$	4877	*MAT*a *ura3-52 lys2-801_amber ade2-101_ochre trp1-Δ63 his3-Δ200 leu2-Δ1 sam50::ade2* [pFL39-$Sam50Cf_{C482}$]	*sam50*Δ	Plasmid shuffling	This study
$Sam50_{C484}$	4878	*MAT*a *ura3-52 lys2-801_amber ade2-101_ochre trp1-Δ63 his3-Δ200 leu2-Δ1 sam50::ade2* [pFL39-$Sam50Cf_{C484}$]	*sam50*Δ	Plasmid shuffling	This study
$Sam50_{C478/C126}$	5060	*MAT*a *ura3-52 lys2-801_amber ade2-101_ochre trp1-Δ63 his3-Δ200 leu2-Δ1 sam50::ade2* [pFL39-$Sam50Cf_{C478/C126}$]	*sam50*Δ	Plasmid shuffling	This study
$Sam50_{C478/C127}$	5061	*MAT*a *ura3-52 lys2-801_amber ade2-101_ochre trp1-Δ63 his3-Δ200 leu2-Δ1 sam50::ade2* [pFL39-$Sam50Cf_{C478/C127}$]	*sam50*Δ	Plasmid shuffling	This study
$Sam50_{C478/C128}$	5062	*MAT*a *ura3-52 lys2-801_amber ade2-101_ochre trp1-Δ63 his3-Δ200 leu2-Δ1 sam50::ade2* [pFL39-$Sam50Cf_{C478/C128}$]	*sam50*Δ	Plasmid shuffling	This study
$Sam50_{C478/C129}$	5063	*MAT*a *ura3-52 lys2-801_amber ade2-101_ochre trp1-Δ63 his3-Δ200 leu2-Δ1 sam50::ade2* [pFL39-$Sam50Cf_{C478/C129}$]	*sam50*Δ	Plasmid shuffling	This study
$Sam50_{C478/C130}$	5064	*MAT*a *ura3-52 lys2-801_amber ade2-101_ochre trp1-Δ63 his3-Δ200 leu2-Δ1 sam50::ade2* [pFL39-$Sam50Cf_{C478/C130}$]	*sam50*Δ	Plasmid shuffling	This study
$Sam50_{C480/C126}$	4879	*MAT*a *ura3-52 lys2-801_amber ade2-101_ochre trp1-Δ63 his3-Δ200 leu2-Δ1 sam50::ade2* [pFL39-$Sam50Cf_{C480/C126}$]	*sam50*Δ	Plasmid shuffling	This study
$Sam50_{C480/C127}$	4880	*MAT*a *ura3-52 lys2-801_amber ade2-101_ochre trp1-Δ63 his3-Δ200 leu2-Δ1 sam50::ade2* [pFL39-$Sam50Cf_{C480/C127}$]	*sam50*Δ	Plasmid shuffling	This study
$Sam50_{C480/C128}$	4881	*MAT*a *ura3-52 lys2-801_amber ade2-101_ochre trp1-Δ63 his3-Δ200 leu2-Δ1 sam50::ade2* [pFL39-$Sam50Cf_{C480/C128}$]	*sam50*Δ	Plasmid shuffling	This study
$Sam50_{C480/C129}$	4882	*MAT*a *ura3-52 lys2-801_amber ade2-101_ochre trp1-Δ63 his3-Δ200 leu2-Δ1 sam50::ade2* [pFL39-$Sam50Cf_{C480/C129}$]	*sam50*Δ	Plasmid shuffling	This study
$Sam50_{C480/C130}$	4883	*MAT*a *ura3-52 lys2-801_amber ade2-101_ochre trp1-Δ63 his3-Δ200 leu2-Δ1 sam50::ade2* [pFL39-$Sam50Cf_{C480/C130}$]	*sam50*Δ	Plasmid shuffling	This study

$Sam50_{C482/C126}$	5065	*MAT*a *ura3-52 lys2-801_amber ade2-101_ochre trp1-Δ63 his3-Δ200 leu2-Δ1 sam50::ade2* [pFL39-$Sam50Cf_{C482/C126}$]	*sam50*Δ	Plasmid shuffling	This study
$Sam50_{C482/C127}$	5066	*MAT*a *ura3-52 lys2-801_amber ade2-101_ochre trp1-Δ63 his3-Δ200 leu2-Δ1 sam50::ade2* [pFL39-$Sam50Cf_{C482/C127}$]	*sam50*Δ	Plasmid shuffling	This study
$Sam50_{C482/C128}$	5067	*MAT*a *ura3-52 lys2-801_amber ade2-101_ochre trp1-Δ63 his3-Δ200 leu2-Δ1 sam50::ade2* [pFL39-$Sam50Cf_{C482/C128}$]	*sam50*Δ	Plasmid shuffling	This study
$Sam50_{C482/C129}$	5068	*MAT*a *ura3-52 lys2-801_amber ade2-101_ochre trp1-Δ63 his3-Δ200 leu2-Δ1 sam50::ade2* [pFL39-$Sam50Cf_{C482/C129}$]	*sam50*Δ	Plasmid shuffling	This study
$Sam50_{C482/C130}$	5069	*MAT*a *ura3-52 lys2-801_amber ade2-101_ochre trp1-Δ63 his3-Δ200 leu2-Δ1 sam50::ade2* [pFL39-$Sam50Cf_{C482/C130}$]	*sam50*Δ	Plasmid shuffling	This study
YPH499 (WT, Fig. 6, S6, S7)	1501	*MAT*a *ura3-52 lys2-801_amber ade2-101_ochre trp1-Δ63 his3-Δ200 leu2-Δ1*	–	–	*67*
Gal1Sam50	2396	*MAT*a *ura3-52 lys2-801_amber ade2-101_ochre trp1-Δ63 his3-Δ200 leu2* $SAM50_{pr}$*::*$SAM50_{pr}$*-HIS3-*$Gal1_{pr}$	YPH499	Homologous recombination (Template 1439, Primers R2 + F4)	*13*
Sam50↓	3988	*MAT*a *ura3-52 lys2-801_amber ade2-101_ochre trp1-Δ63 his3-Δ200 leu2* $SAM50_{pr}$*::*$SAM50_{pr}$*-HIS3-*$Gal1_{pr}$ [pFL39]	Gal1Sam50	Growth on selective medium	This study
Sam50↓+WT	3989	*MAT*a *ura3-52 lys2-801_amber ade2-101_ochre trp1-Δ63 his3-Δ200 leu2* $SAM50_{pr}$*::*$SAM50_{pr}$*-HIS3-*$Gal1_{pr}$ [pFL39-Sam50]	Gal1Sam50	Growth on selective medium	This study
Sam50↓+ $Sam50_{\Delta loop6}$	3990	*MAT*a *ura3-52 lys2-801_amber ade2-101_ochre trp1-Δ63 his3-Δ200 leu2* $SAM50_{pr}$*::*$SAM50_{pr}$*-HIS3-*$Gal1_{pr}$ [pFL39-$Sam50_{\Delta loop6}$]	Gal1Sam50	Growth on selective medium	This study

Table S4. List of antibodies used in this study.

Antigen	Dilution	Number
Cox4	1:500 TBS + 5% milk	GR578-5
Cyc1	1:1000 TBS + 5% milk	GR541-6
Dic1	1:200 TBS + 5% milk	GR2055-2
Mdm10	1:250 TBS + 5% milk	GR1145-3
Om45	1:100 TBS-T + 5% milk	GR1391-4
Por1	1:1000 TBS + 5% milk	94D
Sam50	1:250 TBS + 5% milk	312-6
Sam37	1:500 TBS-T + 5% milk	161-6
Sam35	1:250 TBS + 5% milk	GR551-7
Tim44	1:1000 TBS 5% milk	127-2
Tim23	1:500 TBS + 5% milk	133-9
Tim22	1:500 TBS + 5% milk	164-6
Tim10	1:500 TBS-T + 5% milk	217-3
Tom70	1:250 TBS + 5% milk	GR657-5
Tom40	1:500 TBS-T + 5% milk	168-4
Tom22	1:5000 TBS + 5% milk	GR3227-2
Tom20	1:5000 TBS + 5% milk	GR3225-7
Tom7	1:250 TBS + 5% milk	230-13
Tom5	1:500 TBS + 5% milk	GR3420-7

Model S1. Model of Sam50 β-barrel domain (PDB format).

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