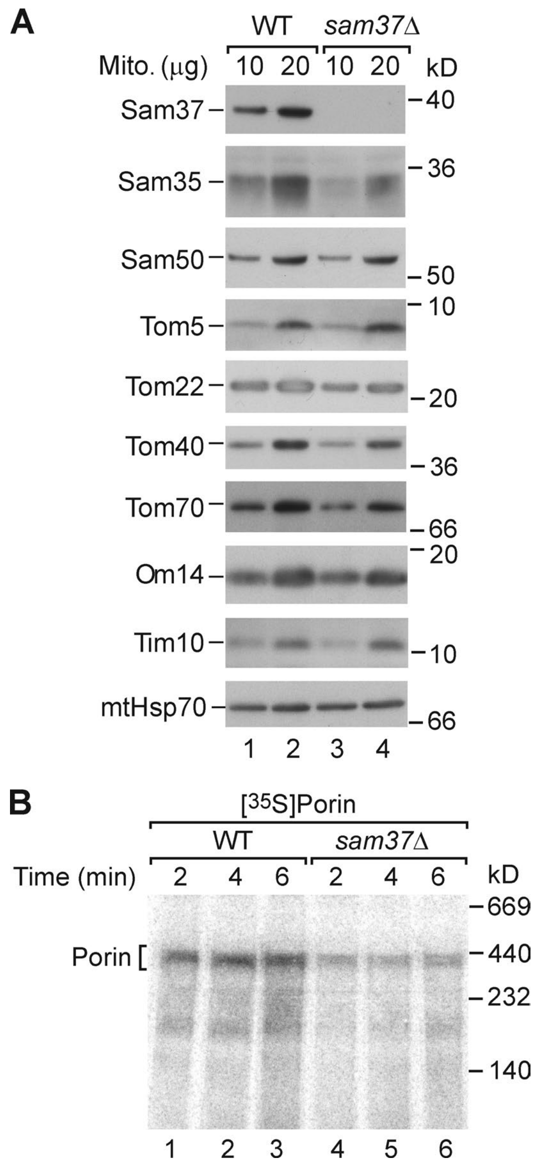

Figure S1. **Steady-state levels of mitochondrial proteins and Porin assembly in *sam37*Δ yeast grown at low temperature.** (A) Wild-type (WT) and *sam37*Δ yeast cells were grown at 19°C. Mitochondria were isolated (microgram, protein amount) and analyzed by SDS-PAGE and immunodetection with the indicated antisera. (B) ^{35}S-labeled Porin was imported into wild-type or *sam37*Δ mitochondria for the indicated periods. Mitochondria were solubilized in digitonin and analyzed by blue native electrophoresis and autoradiography.

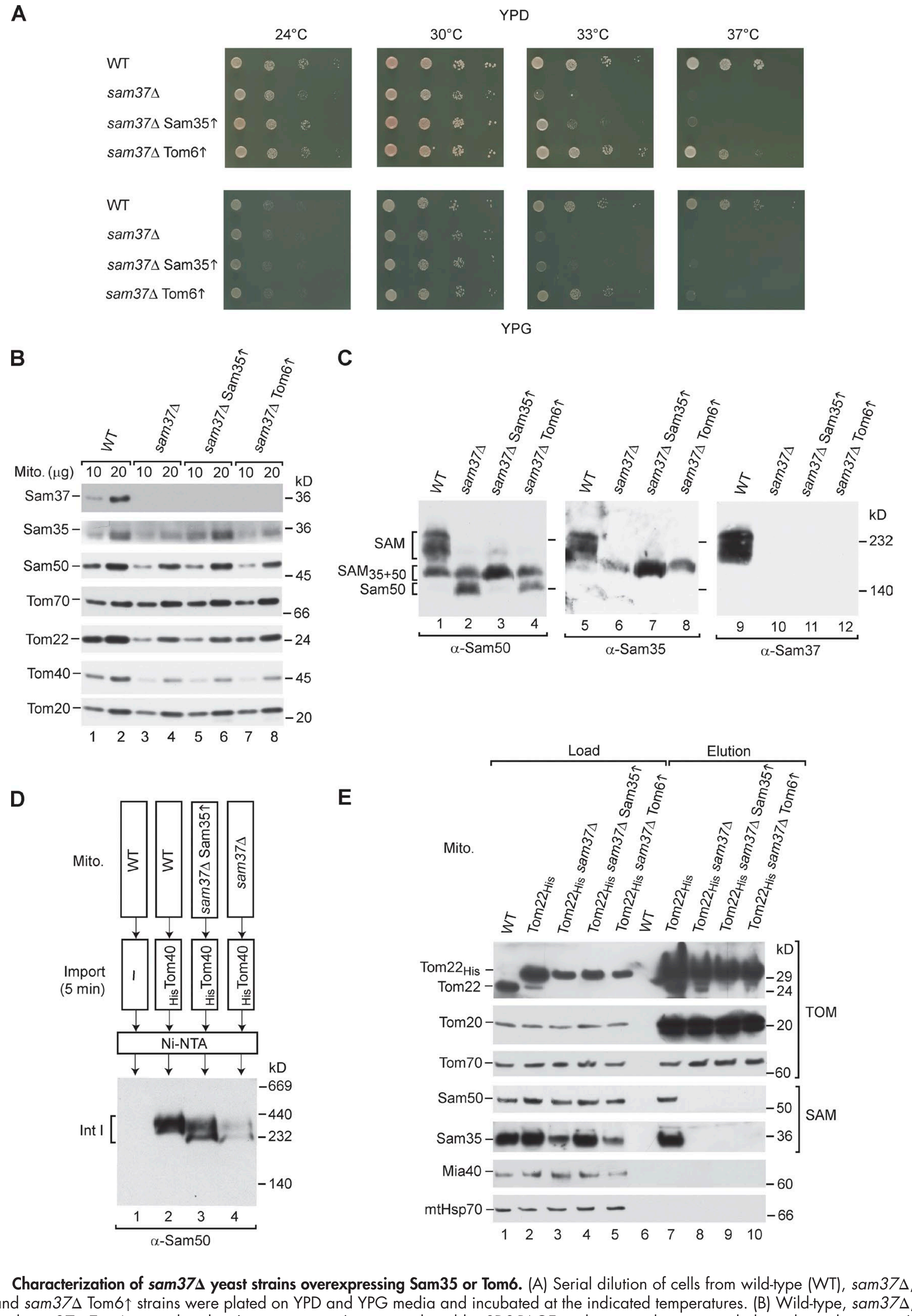

Figure S2. **Characterization of *sam37Δ* yeast strains overexpressing Sam35 or Tom6.** (A) Serial dilution of cells from wild-type (WT), *sam37Δ*, *sam37Δ* Sam35↑, and *sam37Δ* Tom6↑ strains were plated on YPD and YPG media and incubated at the indicated temperatures. (B) Wild-type, *sam37Δ*, *sam37Δ* Sam35↑, and *sam37Δ* Tom6↑ mitochondria (protein amount) were analyzed by SDS-PAGE and immunodetection with the indicated antisera (cells were grown at 19°C). (C) Wild-type, *sam37Δ*, *sam37Δ* Sam35↑, and *sam37Δ* Tom6↑ mitochondria were lysed with digitonin and analyzed by blue native electrophoresis and immunodetection with the indicated antisera. SAM_{35+50}, SAM subcomplex formed by Sam35 and Sam50. (D) Recombinant amounts of $_{His}$Tom40 were imported into wild-type, *sam37Δ*, and *sam37Δ* Sam35↑ mitochondria where indicated. Mitochondria were lysed with digitonin and incubated with Ni-NTA agarose. Elution fractions were analyzed by blue native electrophoresis and immunodetection with antiserum directed against Sam50. (E) Wild-type, $Tom22_{His}$, $Tom22_{His}$ *sam37Δ*, $Tom22_{His}$ *sam37Δ* Sam35↑, and $Tom22_{His}$ *sam37Δ* Tom6↑ mitochondria were lysed and incubated with Ni-NTA agarose. Proteins in load and elution fractions were monitored by SDS-PAGE and immunodetection with the indicated antisera. Load, 3%; elution, 100%.

Table S1. ***S. cerevisiae* strains used in this study**

Strain	Genotype	Number	Reference
YPH499 WT	*MATa ade2-101 his3-Δ200 leu2-Δ1 ura3-52 trp1-Δ63 lys2-801*	1,501	Sikorski and Hieter, 1989
sam37Δ	*MATa ade2-101 his3-Δ200 leu2-Δ1 ura3-52 trp1-Δ63 lys2-801 sam37::LEU2*	1,271	Ryan et al., 1999
Tom22$_{His}$	*MATa ade2-101 his3-Δ200 leu2-Δ1 ura3-52 trp1-Δ63 lys2-801 tom22::TOM22*$_{His}$*-HIS3*	1,456	Meisinger et al., 2001
tom22Δ Tom22↑	*MATa ade2-101 his3-Δ200 leu2-Δ1 ura3-52 trp1-Δ63 lys2-801 tom22::HIS3 pYep352-MET25p-Tom22-CYC1t (2micron, URA3)*	2,281	Qiu et al., 2013
Tom22(N55C)$_{His}$	*MATa ade2-101 his3-Δ200 leu2-Δ1 ura3-52 trp1-Δ63 lys2-801 tom22::HIS3 pFL39-Tom22(C55)*$_{His}$ *(CEN, TRP1)*	3,855	Qiu et al., 2013
Tom22(K66C)$_{His}$	*MATa ade2-101 his3-Δ200 leu2-Δ1 ura3-52 trp1-Δ63 lys2-801 tom22::HIS3 pFL39-Tom22(K66C)*$_{His}$ *(CEN, TRP1)*	4,177	This study
Tom22(ΔIMS)$_{His}$	*MATa ade2-101 his3-Δ200 leu2-Δ1 ura3-52 trp1-Δ63 lys2-801 tom22::HIS3 pFL39-Tom22(ΔIMS)*$_{His}$ *(CEN, TRP1)*	4,401	This study
Tom22(ΔN)$_{His}$	*MATa ade2-101 his3-Δ200 leu2-Δ1 ura3-52 trp1-Δ63 lys2-801 tom22:: HIS3 pFL39-Tom22(Δ4-54)*$_{His}$ *(CEN, TRP1)*	4,400	This study
Tom22$_{His}$ *sam37Δ*	*MATa ade2-101 his3-Δ200 leu2-Δ1 ura3-52 trp1-Δ63 lys2-801 sam37::LEU2 tom22::TOM22*$_{His}$*-HIS3*	4,237	This study
sam37Δ Sam35↑	*MATa ade2-101 his3-Δ200 leu2-Δ1 ura3-52 trp1-Δ63 lys2-801 sam37::LEU2 pRS426-Sam35 (2micron, URA3)*	4,759	This study
sam37Δ Tom6↑	*MATa ade2-101 his3-v200 leu2-Δ1 ura3-52 trp1-Δ63 lys2-801 sam37::LEU2 pRS426-Tom6 (2micron, URA3)*	4,760	This study
WT YPH499 pRS426	*MATa ade2-101 his3-Δ200 leu2-Δ1 ura3-52 trp1-Δ63 lys2-801 pRS426 (2micron, URA3)*	4,761	This study
Tom22$_{His}$ pRS426	*MATa ade2-101 his3-Δ200 leu2-Δ1 ura3-52 trp1-Δ63 lys2-801 tom22::TOM22*$_{His}$*-HIS3 pRS426 (2micron, URA3)*	4,762	This study
Tom22$_{His}$ *sam37Δ* Sam35↑	*MATa ade2-101 his3-Δ200 leu2-Δ1 ura3-52 trp1-Δ63 lys2-801 sam37::LEU2 tom22::TOM22*$_{His}$*-HIS3 pRS426-Sam35 (2micron, URA3)*	4,763	This study
Tom22$_{His}$ *sam37Δ* Tom6↑	*MATa ade2-101 his3-Δ200 leu2-Δ1 ura3-52 trp1-Δ63 lys2-801 sam37::LEU2 tom22::TOM22*$_{His}$*-HIS3 pRS426-Tom6 (2micron, URA3)*	4,764	This study

WT, wild type.

Table S2. **List of antibodies (rabbit antisera) used in this study**

Antibodies directed against	Dilution	Number
S. cerevisiae Atp4 (LQQSISEIEQLLSKLK)	1:1,000 TBS + 5% milk	GR1970-5
S. cerevisiae Cox4 (ARLELLGKLEGIDVFDTKP)	1:1,000 TBS + 5% milk	GR 578-4
E. coli GroEL (isolated full-length protein; antiserum detects *S. cerevisiae* Hsp60)	1:1,000 TBS-T + 5% milk	170 (60)
S. cerevisiae Mia40 (purified full-length protein)	1:1,000 TBS + 5% milk	315-7
S. cerevisiae mtHsp70 (purified full-length protein)	1:2,000 TBS + 5% milk	119-3 (39)
S. cerevisiae Om14 (LDGIISKKYYSRYDKK)	1:4,000 TBS + 5% milk	GR 3040-6
S. cerevisiae Sam35 (KELVQFAQDTLKNFVQ)	1:100 TBS (affinity purified antibodies)	551-7
S. cerevisiae Sam37 (SNNLEQRDPQFRE)	1:50 TBS (affinity purified antibodies)	161-8
S. cerevisiae Sam50 (purified protein comprising AA 1–174)	1:50 TBS + 0.1% Tween (affinity purified antibodies)	312-17
S. cerevisiae Tim10 (GENMQKMGQSFNAAGKF)	1:250 TBS + 5% milk	GR 2040-4
S. cerevisiae Tom5 (MFGLPQQEVSEE)	1:500 TBS + 5% milk	GR 3419-10
S. cerevisiae Tom7 (purified full-length protein)	1:250 TBS + 5% milk	230-10
S. cerevisiae Tom20 (purified protein comprising AA 32–183)	1:10,000 TBS + 5% milk	GR 3225-7
S. cerevisiae Tom22 (purified protein comprising AA 1–97)	1:10,000 TBS + 5% milk	GR 3227-2
S. cerevisiae Tom40 (purified full-length protein)	1:500 TBS + 5% milk	168-5
S. cerevisiae Tom70 (purified protein comprising AA 247–390)	1:500 TBS + 5% milk	GR 657-3

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