SUPPLEMENTAL DATA

EXPERIMENTAL PROCEDURES

Circular dichroism spectroscopy

Proteins were dialyzed against 10 mM sodium/potassium phosphate buffer, pH 7.4 and diluted to 0.1 mg/ml. Spectra were recorded in three replicates at 10 °C from 260 nm to 180 nm, with a 1 nm step size. Spectra were deconvoluted with the Dichroweb server using the CDSSTR method with reference set 3 (2, 3).

Yeast strains and plasmids

Yeast strains were haploid descendants of the wild-type strain DF5. Standard protocols were followed for preparation of yeast media, yeast sporulation and tetrad dissection (4). The lithium–acetate method was used to transform yeast cells (5).

To construct plasmid pFZ37 (Yos9), the PCR product obtained using yeast genomic DNA of YWO1 (*trp1-1(am), his3-*Δ*200, ura3-52, lys2-801, leu2-3,-112*) with the forward primer FZ62, AGCTC GAGGT ATAGC GTCTT TCGCA TCATC, and reverse primer FZ63, TAGGA TCCGA TAACC GATGA CTTGG CAAG, was ligated into pGEM-T (Promega, Madison, WI, USA), digested with *Xho*I and *BamH*I and ligated into *Xho*I and *BamH*I digested pRS416. Site-directed mutagenesis was performed in a QuikChange Mutagenesis process to construct plasmids pFZ54 (Yos9 L393A) and pFZ55 (Yos9 L393A/N380A).

Co-immunoprecipitation

Yeast cells were grown in SD medium (supplemented with complete amino acid or –URA – mix) at 30 °C to logarithmic phase. An equivalent of 100 OD cells was harvested and washed once with ice-cold water supplemented with 1 mM PMSF. Cells were disrupted using glass beads in 400 µl IP32 buffer (50 mM HEPES (NaOH), pH 7.2, 20 mM NaCl, 125 mM KOAc, pH 7.5, 2 mM MgOAc, 1 mM EDTA, 10 µM $CaCl_2$, 3% glycerol) containing 1 mM PMSF. 1.2 ml IP32 was added. Cellular debris was removed by centrifugation for 1 min at 5,000×*g*. Microsomes were collected by centrifugation at 20,000×*g* at 4 °C for 20 min and solubilized in 1.2 ml IP32 plus 0.5% Nonidet P-40 (NP40). Unsoluble material was removed by centrifugation at 20,000×*g* at 4 °C for 10 min. HA-Hrd3 was precipitated from the supernatant with 2 µl anti-HA antibody and 20 µl protein A Sepharose beads (GE Healthcare) at 4 °C overnight. Beads were washed three times with 1 ml IP32 plus 0.5% NP40, and bound proteins were eluted with 30 µl SDS sample buffer. Proteins were analyzed by SDS-PAGE and immunoblotting, using monoclonal HA antibody and Yos9 antiserum.

Pulse-chase experiments

Pulse-chase experiments were performed as described (6). Briefly, exponentially grown cells were pulse-labeled at 30 °C in synthetic dropout complete medium with Express^{35}S labeling mix (Perkin Elmer, Waltham, MA, USA) for 10 min, and samples were taken at indicated time points during the chase. CPY* was immunoprecipitated from total cell lysates and analyzed by SDS-PAGE autoradiography after endoglycosidase F digestion.

FIGURES

FIGURE S1. Surface view of the Yos9 DD structure. *A.* Top: Overall fold superimposed with a semi-transparent surface. Bottom: Electrostatic surface potential shown in the range of +10 kT (blue) to -10 kT (red), calculated with APBS (1). The hydrophobic groove is marked with an asterisk. *B.* In one molecule of the asymmetric unit, the hydrophobic groove accommodates the elongated N-terminus of a neighboring molecule in the crystal lattice, here represented with a blue surface.

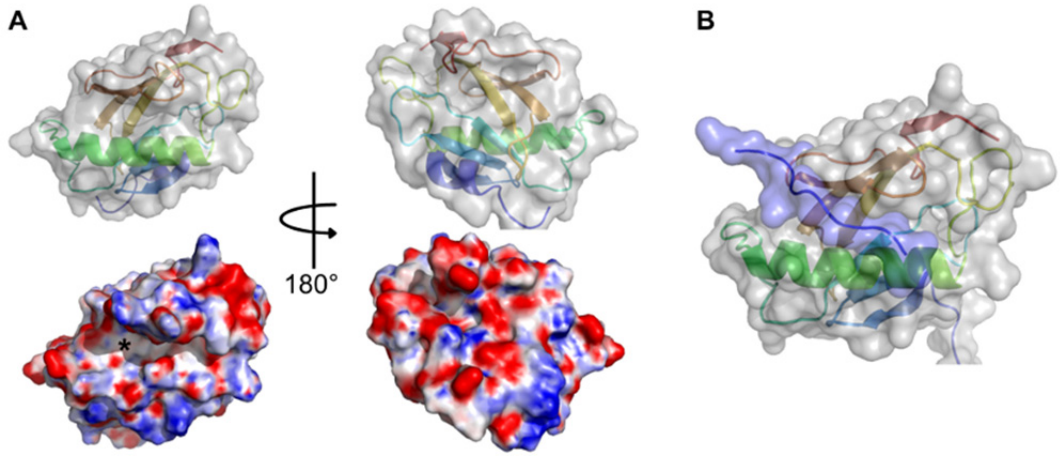


FIGURE S2. CD spectra of the wildtype Yos9 DD ($Yos9_{266-424}$) and variants (L393A, N380A) thereof. Solid, wildtype; dotted, L393A; dashed, N380A.

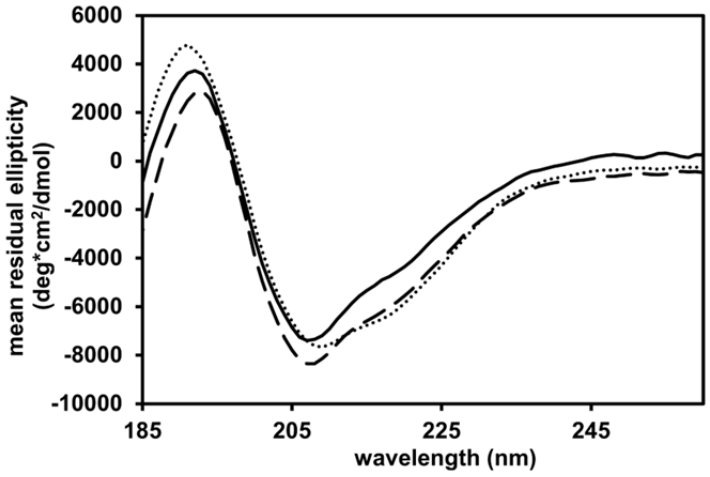


FIGURE S3. *A.* SDS-PAGE analysis of the purified Yos9 truncation variants used in this study. *B.* CD spectra of the truncation variants. Left: solid black, $Yos9_{24-539}$; solid gray, $Yos9_{90-262}$; dashed black, $Yos9_{24-262}$; dashed gray, $Yos9_{24-424}$. Right: solid black, $Yos9_{90-424}$; solid gray, $Yos9_{266-539}$; dashed black, $Yos9_{266-424;}$ dashed gray, $Yos9_{24-90}$.

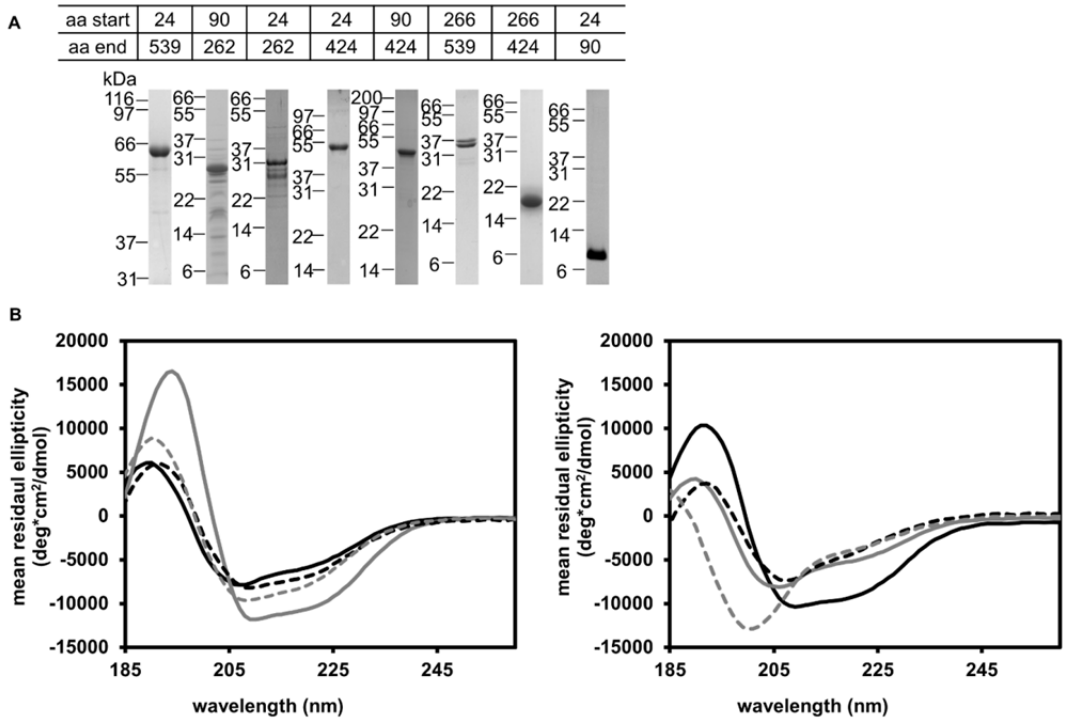


FIGURE S4. Pull-down experiment between Yos9 proteins and Hrd3. $Yos9_{266-424}$ (DD) and $Yos9_{24-539}$ (full-length) were purified from recombinant *E. coli* cells as heptahistidine fusion proteins and incubated with a microsomal extract from a *Yos9* knockout strain expressing HA-tagged Hrd3 after immobilization on a Co^{2+}-affinity resin. The control (2 lanes at the left) shows background binding of HA-Hrd3 to the matrix. Full-length Yos9, but not the Yos9 DD binds HA-Hrd3 as indicated by enrichment in the eluate (e) over the flowthrough (ft) of the affinity column.

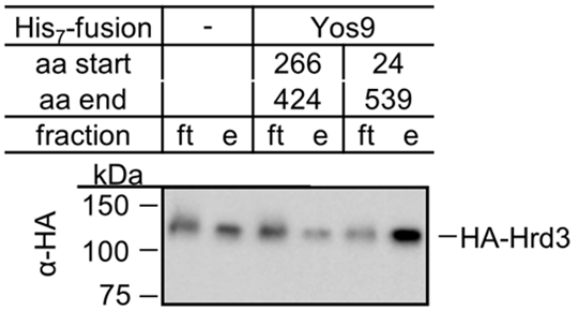
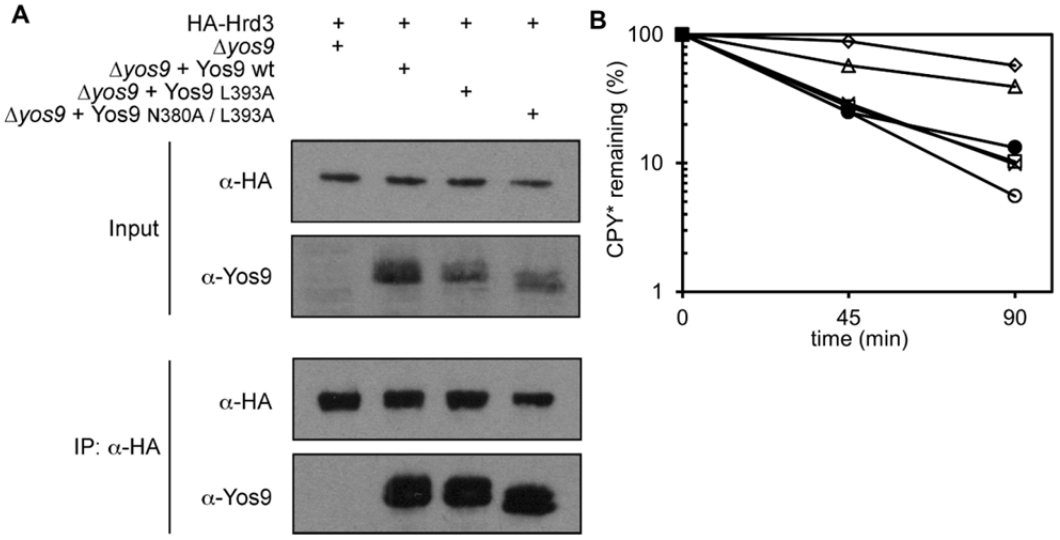


FIGURE S5. *In vivo* experiments with dimerization deficient Yos9 mutants. *A.* Co-immunoprecipitation. Mutant and wildtype Yos9 was co-immunoprecipitated from microsomal preparations with HA-tagged Hrd3. *B.* CPY* degradation, measured by quantification of pulse-chase experiments. Diamonds, *ΔHrd3*; triangles, *Δyos9*; open circles, wildtype; closed circles, *Δyos9* + Yos9; squares, *Δyos9* + Yos9 L393A; crosses, *Δyos9* + Yos9 N380A / L393A double mutant.



TABLES

TABLE S1. Properties of the Yos9 DD self-association interfaces and assemblies. Surface areas assume the N-termini to be present in the helical conformation. ΔG^{i}, solvation free energy gain upon formation of the interface; ΔG^{int}, solvation free energy gain upon formation of the assembly; ΔG^{diss}, free energy of assembly dissociation with positive values indicating thermodynamic stability; $T\Delta S^{diss}$, entropy change upon assembly dissociation.

Multimeric state			Dimer	Trimer
Interface	interfacing	residues	49	31
		atoms	180	116
	area, Å^2	buried	884	504
	ΔG^{i}	kcal/mol	-7.3	-5.5
		P value	0.278	0.192
	no. of hydrogen bonds		20	4
	no. of salt bridges		4	0
Assembly	area, Å^2	surface	13,939	20,538
		buried	1,769	3,024
	ΔG^{int}	kcal/mol	-7.3	-16.6
	ΔG^{diss}	kcal/mol	4.9	-2.0
	$T\Delta S^{diss}$	kcal/mol	11.9	23.9

TABLE S2. Interfacing residues in the dimeric assembly of the Yos9 DD. H: hydrogen bond; S: salt bridge; ASA: accessible surface area, Å²; BSA: buried surface area, Å²; ||||: buried area percentage, one bar per 10%; ΔG^{i}: solvation energy effect, kcal/mol.

ChainA					**ChainB**				
residue	**HS**	**ASA**	**BSA**	**ΔG^{i}**	**residue**	**HS**	**ASA**	**BSA**	**ΔG^{i}**
Ser347		21.09	0.24|	-0.00	**Ser347**		21.01	1.11|	-0.01
Cys348		5.72	0.63||	0.01	**Cys348**		6.44	0.77||	0.01
Val349		34.16	33.82|||||||||	0.54	**Val349**		36.31	36.14|||||||||	0.58
Leu351		20.08	19.91|||||||||	0.32	**Leu351**		19.56	19.56||||||||||	0.31
Thr364		2.85	2.85||||||||||	0.05	**Thr364**		2.51	2.51||||||||||	0.04
Ser366		28.32	27.74|||||||||	0.02	**Ser366**		30.93	29.70|||||||||	0.09

Asn368	H	50.98	41.56||||||||	-0.33	**Asn368**	H	53.50	37.31||||||	-0.32
Ile369		17.08	0.74|	-0.01	**-**				
Leu370		85.73	30.63||||	0.49	**Leu370**		78.99	26.11||||	0.42
Glu376	HS	108.60	43.29||||	-0.26	**Glu376**	HS	111.36	40.22||||	-0.24
Phe378		119.94	76.05||||||	1.22	**Phe378**		120.32	72.49||||||	1.16
Asn380	H	45.02	28.11||||||	-0.26	**Asn380**	H	45.18	26.18|||||	-0.24
Thr382		120.10	14.01||	-0.15	**Thr382**		115.35	11.11|	-0.13
Phe383		92.82	88.35|||||||||	1.41	**Phe383**		98.54	93.71|||||||||	1.50
Thr384	H	105.99	62.97|||||	-0.17	**Thr384**	H	109.19	65.45|||||	-0.16
Phe385	H	29.34	8.30|||	-0.02	**Phe385**		27.24	7.18|||	-0.01
Asn386	H	94.61	54.48|||||	-0.23	**Asn386**	H	97.20	62.59||||||	-0.27
Asp388	H	122.18	26.55|||	-0.40	**Asp388**	H	122.27	30.50|||	-0.46
Asn389	H	63.45	54.63||||||||	-0.38	**Asn389**	H	64.81	55.23||||||||	-0.42
Gly390		44.61	3.38|	-0.04	**Gly390**		47.45	4.73|	-0.05
Phe392	H	39.29	16.45|||||	-0.16	**Phe392**	H	41.48	16.08||||	-0.16
Leu393	H	133.67	133.51|||||||||	1.50	**Leu393**	H	133.63	133.31|||||||||	1.52
Ser394		53.64	44.36||||||||	0.54	**Ser394**		55.92	43.73|||||||	0.51
Tyr395		93.60	35.36||||	0.09	**Tyr395**		103.23	33.23||||	0.12
Lys396	HS	141.91	35.58|||	-0.10	**Lys396**	HS	146.65	36.46|||	-0.21

TABLE S3. Interfacing residues in the trimeric assembly of the Yos9 DD. H: hydrogen bond; ASA: accessible surface area, Å²; BSA: buried surface area, Å²; ||||: buried area percentage, one bar per 10%; ΔG^i: solvation energy effect, kcal/mol.

$ChainB_1$					**$ChainB_2$**				
residue	**H**	**ASA**	**BSA**	**ΔG^i**	**residue**	**H**	**ASA**	**BSA**	**ΔG^i**
Ile266		89.93	12.60||	-0.13	**Ile271**		127.64	19.57||	0.31
Gly267		82.94	48.58||||||	-0.03	**Asp272**		116.80	4.46|	-0.05
Ser268		82.99	38.73|||||	0.57	**Ile274**		76.24	24.05||||	0.13
Asn269	H	45.54	39.28||||||||	-0.24	**Thr275**	H	90.62	86.69|||||||||	0.66

Ser270		29.90	0.24|	-0.00	**Lys276**		87.70	27.93||||	0.41
Ile271		127.64	37.35|||	0.60	**Glu278**		65.09	32.01|||||	-0.16
Ile274		76.24	44.86||||||	0.72	**Arg290**		93.57	46.89||||||	-0.49
Pro279		47.90	23.84|||||	0.09	**Pro291**	H	15.60	4.55|||	-0.05
Ile280		31.80	1.67|	0.03	**Phe292**	H	124.75	119.57||||||||||	1.47
Phe281		101.68	44.80|||||	0.62	**Asn293**		78.37	61.92||||||||	0.24
Ser284		39.14	0.16|	0.00	**Thr294**		129.09	67.58||||||	0.68
Gly285		18.40	13.63||||||||	0.11	**Asp295**	H	131.70	25.60||	-0.23
Tyr287	H	57.92	56.08||||||||||	-0.01	**Glu296**		68.10	9.55||	-0.14
Met301		3.52	3.52||||||||||	0.06					
Thr303	H	36.99	33.05|||||||||	0.03					
Asp304		34.97	16.77|||||	0.14					
Asn305	H	120.47	46.42||||	-0.36					
Met357		20.77	15.93||||||||	0.55					

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