Supplementary information

Methods

Evolutionary sequence analysis

Homologs of Jac1 were identified by TBLASTN searches with *S. cerevisiae* sequence as query against the completed genome sequence for each of the species listed in the text. When available, the refseq protein database was searched by BLASTP. Protein pairwise evolutionary distances were calculated with TREE-PUZZLE (Schmidt et al., 2002) under the Whelan and Goldman (WAG) (Whelan and Goldman, 2001) substitution matrix using 8 rate categories for the gamma distribution and invariable sites estimated from the data set. Model selection was performed according to the Akaike Information Criterion (AIC) values obtained with ProtTest (Abascal et al., 2005).

The Jac1 protein tree was inferred by maximum likelihood. Alignment was guided by structural information and was comprised of the J-domains and the C-terminal domains, save for the interdomain linker and the HPD-S variable loop. Model selection was based on the results obtained from ProTest (Abascal et al., 2005). We used PhyML (Guindon and Gascuel, 2003) under the LG (Le and Gascuel, 2008) model of protein evolution, with site-to-site rate variation modeled on a discrete gamma distribution (four categories for the shape parameter gamma and a proportion of invariant sites estimated from data). Tree topology was optimized by SPR branch swapping, and confidence in branch support was assessed with 100 bootstrap replicates.

Prediction of protein structure

For the homology modeling of proteins three factors influence the quality of the predicted structure: the evolutionary distance between the target and the template, the quality of sequence alignment of target to query protein, and the quality of the target structure (Chakravarty et al., 2005). Two solved structures of Jac1 orthologs, *Escherichia coli* HscB (PDB ID: 1fpo) (Cupp-Vickery and Vickery, 2000) and *Homo sapiens* Jac1 (PDB ID: 3bvo) (Bitto et al., 2008) are available at 1.8 Å and 3.0 Å resolution, respectively. The HscB protein was utilized as a template for modeling. To ensure the best sequence alignment between the target and the template, the target sequence was submitted to the GeneSilico metaserver, which is a gateway to a number of different structure prediction methods. For the actual fold recognition procedure, generates target-template alignments, the metaserver employed the following methods: FFAS03 (Rychlewski et al., 2000), SAM-T02 (Karplus et al., 2003), 3DPSSM (Kelley et al., 2000), INBGU (Fischer, 2000), FUGUE (Shi et al., 2001), mGEN-THREADER (Jones, 1999) and SPARKS (Zhou and Zhou, 2004). Alignments resulting from above calculations were compared and their quality estimated with Pcons5 (Lundstrom et al., 2001). The best scored sequence alignment was chosen and minor refinements, were done manually in the Swiss-PdbViewer (http://www.expasy.org/spdbv) (Guex and Peitsch, 1997), based on predictions of secondary structure performed with PSIPRED (McGuffin et al., 2000), PROFsec (Rost et al., 2004), Prof (Ouali and King, 2000), SABLE (Adamczak et al., 2004), JNET (Cuff and Barton, 2000), JUFO (Meiler and Baker, 2003), SAM-T02 (Karplus et al., 2003), taking into account natively disordered region recognition conducted with PONDR (Romero et al., 2004), DISOPRED (Ward et al., 2004), and solvent accessibility calculated with SABLE (Adamczak et al., 2004) and JPRED (Cuff et al., 1998). Protein structure models were calculated with MODELLER (Fiser and Sali, 2003) and their potential accuracy was evaluated with MetaMQAP (Pawlowski et al., 2008).

To assess the validity of our modeling protocol, we predicted the structure of human Jac1, using the existing *E. coli* structure as a template (Fig. S2). A comparison of the modeled and experimentally determined structures revealed an overall root mean square deviation between the matched backbone atoms of 1.3 Å, indicating the high level of similarity between them, hence high accuracy of the model (Fig. S2B). Having validated our approach, we proceeded to analyze $Jac1_{Sc}$ and $Jac1_{Yl}$. According to MetaMQAP (Pawlowski et al., 2008), the models are predicted to exhibit the root mean square deviation of 2.4 Å and 3.3 Å to the true structures for $Jac1_{Sc}$ and $Jac1_{Yl}$, respectively. Hence they could be used as medium-resolution working models for functional analyses.

Biochemical assays

Glycerol gradient centrifugation. Glycerol gradient centrifugation was carried out as described in (Dutkiewicz et al., 2003). Purified proteins ($Isu1_{Sc}$, $Jac1_{Sc}$, and $Jac1_{Yl}$), alone or in combination, were placed in a reaction mixture (80 µl) in buffer G (40 mM HEPES-KOH, pH 7.5, 100 mM KCl, 1 mM dithiothreitol, 10 mM $MgCl_2$, 5% (v/v) glycerol), and incubated for 10 min at 25 ^oC. Then, 70 µl of this mixture was loaded onto a 3-ml linear 15-35% (v/v) glycerol gradient prepared in buffer G, and centrifuged at 2 ^oC in a Beckman SW60 rotor for 28 h at 46,000 rpm. Fractions (130 µl each) were collected from the top of the tube and their protein contents were analyzed by SDS-PAGE followed by silver staining.

ATPase activity of Ssc1 and Ssq1. The release of radioactive inorganic phosphate from [γ-^{32}P]ATP was measured as described in (Viitanen et al., 1990). Reaction mixtures contained $Ssc1_{Sc}$ or $Ssq1_{Sc}$ at 0.5 µM, $Isu1_{Sc}$ at 10 µM, $Mge1_{Sc}$ at 0.5 µM and the indicated Jac1 protein at the indicated concentrations in buffer A (40 mM HEPES-KOH, pH 7.5, 100 mM KCl, 1 mM dithiothreitol, 10 mM $MgCl_2$, 10% (v/v) glycerol). Reactions were initiated by the addition of ATP (2µCi, DuPont NEG-003H, 3000 Ci/mmol) to a final concentration of 120 µM. Incubation was carried out at 25 ^oC and the reaction terminated by the removal (at 5, 10, 15 and 20 min after initiation of the reaction) of 20-µl aliquots to an Eppendorfe tube containing 175 µl of 1 M perchloric acid and 1 mM sodium phosphate. After addition of 400 µl of 20 mM ammonium molybdate and 400 µl of isopropyl acetate, samples were mixed and the phases separated by a short centrifugation. An aliquot of the organic phase (150 µl), containing the radioactive orthophosphate-molybdate complex, was removed and radioactivity was determined by liquid scintillation counting. Control reactions lacking protein were included in all experiments.

Legends to supplementary figures

Fig. S1 Alignment of selected Jac1 sequences. The alignment was prepared manually based on predicted structures. Number and position of helices (I-VII) and the inter-domain linker are indicated. Conserved amino acids are highlighted: 9/12 identical in black and 9/12 similar in grey (as defined by BLOSUM 62 matrix). Conserved HPD motif and universally conserved serine in helix III are indicated by asterisk.

Fig. S2 Predicted structures of Jac1 orthologs from *S. cerevisiae* and *Y. lipolytica*. (A) Structures of Jac1 orthologs from *E. coli* (PDB id: 1fpo) and *H. sapiens* (PDB id: 3bvo) obtained by X-ray crystallography. (B) Comparison of experimental structure and model of *H. sapiens* Jac1 homolog. Structure (light) as obtained by X-ray diffraction crystallography at 3 Å resolution (Bitto et al., 2008). Model structure (dark) was obtained by homology-based

computer modeling utilizing the solved structure of *E. coli* Jac1 ortholog (see A) as a template. The calculated RMS value for all backbone atoms was: 1.28 Å for entire protein, 0.9 Å for J-domain fragment, and 2 Å for C-terminus. Residues: 1-71, not present in the *E. coli* protein, were excluded from calculations. (C) Model structures of $Jac1_{Sc}$ and $Jac1_{Yl}$ obtained utilizing the solved structure of *E. coli* Jac1 ortholog as a template.

Fig. S3 Jac1 from *Y. lipolytica* efficiently binds Isu1 from *S. cerevisiae* and this interaction is not a limiting factor in the stimulation of the ATPase activity of $Ssc1_{Sc}$. (A) Sedimentation of Isu1 and Jac1 from *Y. lipolytica* and *S. cerevisiae* (Isu_{Yl}, Isu_{Sc}, $Jac1_{Yl}$ and $Jac1_{Sc}$). Reaction conditions were as described in Suppl. Methods. Fractions collected from the gradient were assayed by SDS-PAGE followed by silver staining. (B-C) Plots representing quantification of protein contents were assessed by densitometry analysis using Quantity One software (Bio-Rad). (D) Stimulation of the $Ssc1_{Sc}$ ATPase activity in the presence of $Isu1_{Sc}$ or Isu_{Yl}. Reaction conditions as described in Suppl. Methods.

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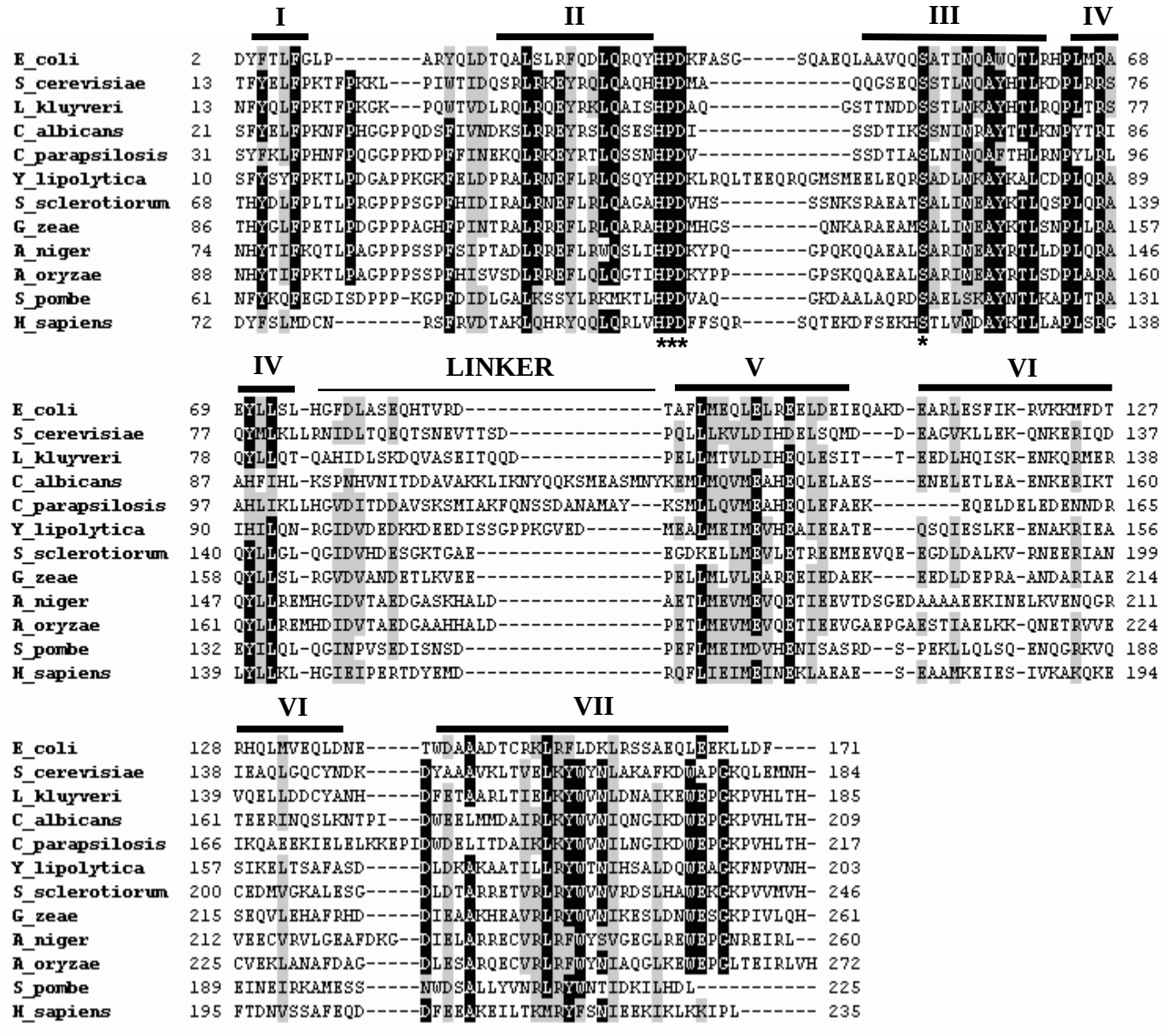
Fig. S1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II
III
IV
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S_cerevisiae 13 TFYELFPKTFPKKL---PIWTIDQSRLRKEYRQLQAQHHPDMA----------QQGSEQSSTLNQAYHTLKDPLRRS 76
L_kluyveri 13 NFYQLFPKTFPKGK---PQWTVDLRQLRQEYRKLQAISHPDAQ----------GSTTNDDSSTLNKAYHTLRQPLTRS 77
C_albicans 21 SFYELFPKNFPHGGPPQDSFIVNDKSLRREYRSLQSESHPDI----------SSDTIKSSNINPAYTTLKNPYTRI 86
C_parapsilosis 31 SYFKLFPHNFPQGGPPKDPFFINEKQLRKEYRTLQSSNHPDV----------SSDTIASLNINQAFTHLRNPYLRL 96
Y_lipolytica 10 SFYSYFPKTLPDGAPPKGKFELDPRALRNEFLRLQSQYHPDKLRQLTEEQRQGMSMEELEQRSADLNKAYKALCDPLQRA 89
S_sclerotiorum 68 THYDLFPLTLPRGPPPSGPFHIDIRALRNEFLRLQAGAHPDVHS--------SSNKSRAEATSALINEAYKTLQSPLQRA 139
G_zeae 86 THYGLFPETLPDGPPPAGHFPINTRALRREFLRLQARAHPDMHGS--------QNKARAEAMSALINEAYKTLSNPLLRA 157
A_niger 74 NHYTIFKQTLPAGPPPSSPFSIPTADLRREFLRWQSLIHPDKYPQ-------GPQKQQAEALSARINEAYRTLLDPLQRA 146
A_oryzae 88 NHYTIFPKTLPAGPPPSSPFHISVSDLRREFLQLQGTIHPDKYPP-------GPSKQQAEALSARINEAYRTLSDPLARA 160
S_pombe 61 NFYKQFEGDISDPPP-KGPFDIDLGALKSSYLRKMKTLHPDVAQ--------GKDAALAQRDSAELSKAYNTLKAPLTRA 131
H_sapiens 72 DYFSLMDCN--------RSFRVDTAKLQHRYQQLQRLVHPDFFSQR-----SQTEKDFSEKHSTLVMDAYKTLLAPLSRG 138
***
*
IV
LINKER
V
VI
E_coli 69 EYLLSL-HGFDLASEQHTVRD------------------TAFLMEQLELREELDEIEQAKD-EARLESFIK-RVKKMFDT 127
S_cerevisiae 77 QYMLKLLRNIDLTQEQTSNEVTTSD--------------PQLLLKVLDIHDELSQMD---D-EAGVKLLEK-QNKERIQD 137
L_kluyveri 78 QYLLQT-QAHIDLSKDQVASEITQQD--------------PELLMTVLDIHEQLESIT---T-EEDLHQISK-ENKQRMER 138
C_albicans 87 AHFIHL-KSPNHVNITDDAVAKKLIKNYQQKSMEASMNYKEMLMQVMEAHEQLELAES----ENELETLEA-ENKERIKT 160
C_parapsilosis 97 AHLIKLLHGVDITDDAVSKSMIAKFQNSSDANAMAY---KSMLLQVMEAHEQLEFAEK--------EQELDELEDENNDR 165
Y_lipolytica 90 IHILQN-RGIDVDEDKKDEEDISSGPPKGVED-------MEALMEIMEVHEAIEEATE----QSQIESLKE-ENAKRIEA 156
S_sclerotiorum 140 QYLLGL-QGIDVHDESGKTGAE------------------EGDKELLMEVLETREEMEEVQE-EGDLDALKV-RNEERIAN 199
G_zeae 158 QYLLSL-RGVDVANDETLKVEE------------------PELLMLVLEAREEIEDAEK----EEDLDEPRA-ANDARIAE 214
A_niger 147 QYLLREMHGIDVTAEDGASKHALD----------------AETLMEVMEVQETIEEVTDSGEDAAAAEEKINELKVENQGR 211
A_oryzae 161 QYLLREMHDIDVTAEDGAAHHALD----------------PETLMEVMEVQETIEEVGAEPGAESTIAELKK-QNETRVVE 224
S_pombe 132 EYILQL-QGINPVSEDISNSD------------------PEFLMEIMDVHENISASRD--S-PEKLLQLSQ-ENQGRKVQ 188
H_sapiens 139 LYLLKL-HGIEIPERTDYEMD------------------RQFLIEIMEINEKLAEAE---S-EAAMKEIES-IVKAKQKE 194
VI
VII
E_coli 128 RHQLMVEQLDNE-----TWDAQADTCRKLRFLDKLRSSAEQLEEKLLDF---- 171
S_cerevisiae 138 IEAQLGQCYNDK-----DYAAQVKLTVELKYVYQLAKAFKDVAPCKQLEMNH- 184
L_kluyveri 139 VQELLDDCYANH-----DFETQARLTIELKYVVVLDNAIKEWEPCKPVHLTH- 185
C_albicans 161 TEERINQSLKNTPI---DWEELMMDAIRLKYVVVIQNGIKDWEPCKPVHLTH- 209
C_parapsilosis 166 IKQAEEKIELELKKEPIDWDELITDAIKLKYVVVILNGIKDWEPCKPVHLTH- 217
Y_lipolytica 157 SIKELTSAFASD-----DLDKQKAATILLRYVTVIHSALDQWEACKFNPVNH- 203
S_sclerotiorum 200 CEDMVGKALESG-----DLDTARRETVRLRYVVVVRDSLHAWEKCKPVVMVH- 246
G_zeae 215 SEQVLEHAFRHD-----DIEAQKHEAVRLRYVVVIKESLDNWESCKPIVLQH- 261
A_niger 212 VEECVRVLGEAFDKG--DIELARRECVRLRFVYSVGEGLREWEPCNREIRL-- 260
A_oryzae 225 CVEKLANAFDAG-----DLESQRQECVRLRFVYVIAQGLKEWEPCLTEIRLVH 272
S_pombe 189 EINEIRKAMESS-----NWDSQLLYVNRLRYVNTIDKILHDL---------- 225
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Fig. S2

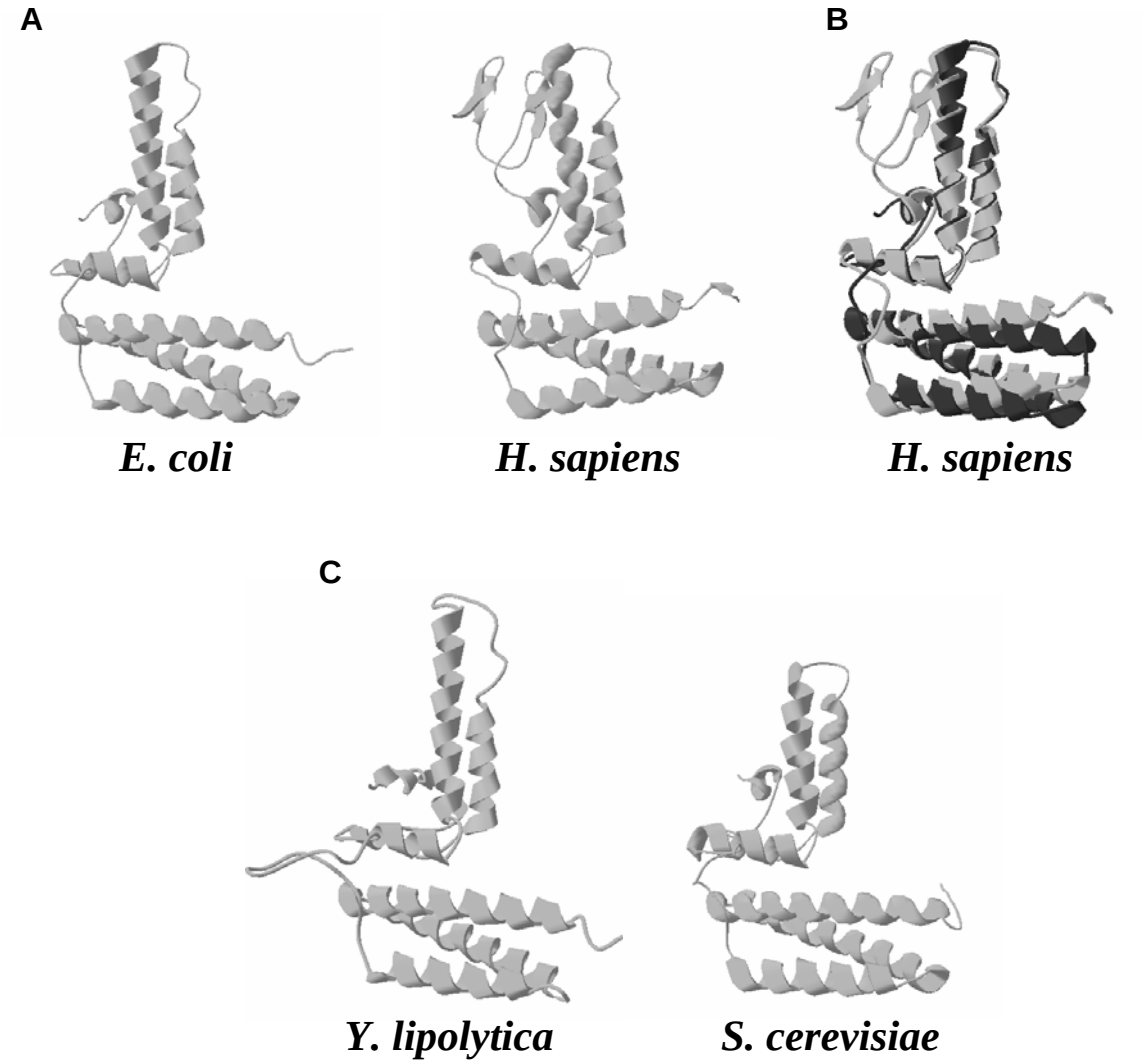
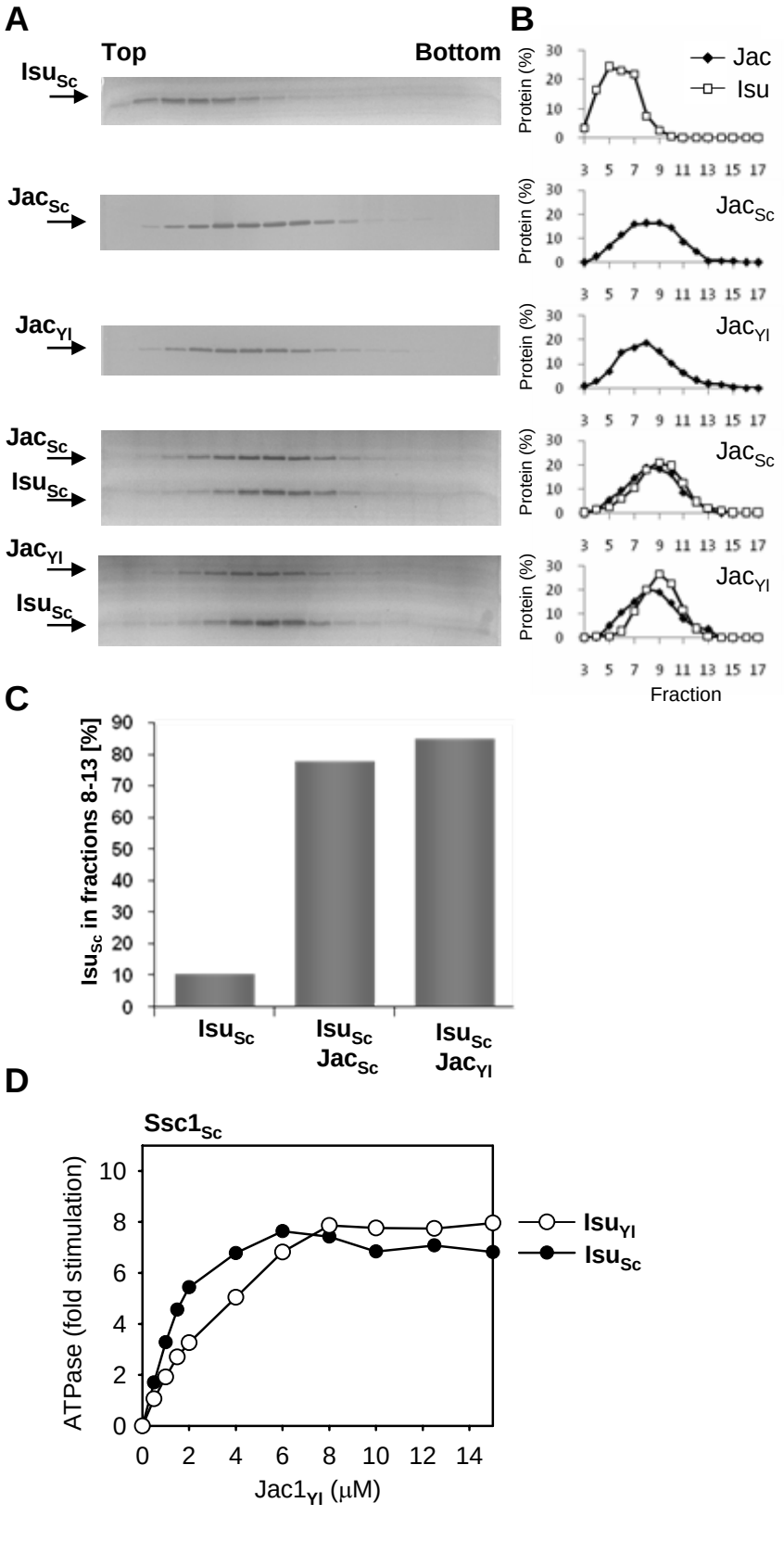


Fig. S3


A
Top
Bottom
Isu$_{Sc}$
Jac$_{Sc}$
Jac$_{Yl}$
Jac$_{Sc}$
Isu$_{Sc}$
Jac$_{Yl}$
Isu$_{Sc}$
B
Jac
Isu
Protein (%)
Jac$_{Sc}$
Jac$_{Yl}$
Fraction
C
Isu$_{Sc}$ in fractions 8-13 [%]
Isu$_{Sc}$
Isu$_{Sc}$ Jac$_{Sc}$
Isu$_{Sc}$ Jac$_{Yl}$
D
Ssc1$_{Sc}$
ATPase (fold stimulation)
Jac1$_{Yl}$ (μM)
Isu$_{Yl}$
Isu$_{Sc}$

Tab. S1 Kinetic parameters of stimulation of ATPase activity by $Jac1_{Yl}$, $Jac1_{Sc}$ and chimerical Jac1 proteins.

Ssc1/Jac1	MS	(S.E.)	$C_{0.5}$	(S.E.)
$Jac1_{Yl}$	8.28	(0.47)	2.16	(0.42)
$Jac1_{Sc}$	3.29	(0.40)	6.71	(1.83)
$Jac1_{Sc}Y23$	10.54	(0.67)	5.72	(0.87)
$Jac1_{Sc}Y23\Delta13$	n.d.*		n.d.*	

Ssq1/Jac1	MS	(S.E.)	$C_{0.5}$	(S.E.)
$Jac1_{Yl}$	4.11	(0.80)	6.14	(1.480)
$Jac1_{Sc}$	10.36	(0.28)	0.050	(0.007)
$Jac1_{Sc}Y23$	9.32	(0.33)	0.054	(0.010)
$Jac1_{Sc}Y23\Delta13$	9.84	(0.17)	1.010	(0.068)

Maximal Stimulation (MS) and Jac1 concentration giving half-maximal stimulation ($C_{0.5}$) were calculated using non-linear regression, as implemented in Sigma-Plot, by fitting Michaelis-Menten hyperbolic equation to data presented at Fig. 2A. Standard error (S.E) of estimated parameter is given for each MS and $C_{0.5}$ value.
*- not determinable.

Table S2
List of species and parameters values from Fig. 3A

Species	D-S *	Distance#
Saccharomyces (11 species)		
Saccharomyces cerevisiae	8	0
Saccharomyces paradoxus	8	0,07
Saccharomyces mikatae	8	0,11
Saccharomyces kudriavzevii	8	0,21
Saccharomyces bayanus	8	0,34
Saccharomyces castelli	11	0,65
Candida glabrata	12	0,99
Lachancea kluyveri	9	1,01
Kluyveromyces lactis	9	1,05
Lachancea waltii	9	1,07
Eremothecium gossypii	8	1,41
Candida (8 species)		
Candida albicans	7	1,60
Loderomyces elongisporus	7	1,96
Candida dubliniensis	7	2,07
Clavispora lusitaniae	2	2,12
Pichia guillermondii	27	2,21
Candida parapsilosis	7	2,15
Debaromyces hansenii	20	2,31
Candida tropicalis	7	2,73
Fusarium (9 species)		
Trichoderma reesei	23	1,55
Gibberella moniliformis	13	1,74
Gibberella zeae	13	1,74
Neurospora crassa	30	1,95
Sclerotinia sclerotiorum	13	1,98
Botryotinia fuckeliana	13	2,25
Magnaporthe grisea	27	2,26
Podospora anserina	17	2,49
Phaeosphaeria nodorum	13	2,71

Species	D-S*	Distance#
Aspergillus (11 species)		
Aspergillus oryzae	14	1,76
Histoplasma capsulatum	14	1,79
Aspergillus nidulans	14	1,97
Coccidioides immitis	14	2,00
Aspergillus clavatus	14	2,02
Aspergillus fumigatus	14	1,99
Aspergillus terreus	14	2,04
Neosartorya fischeri	14	2,05
Aspergillus flavus	14	2,09
Aspergillus niger	14	2,48
Uncinocarpus reesii	14	3,91
Yarrowia (1 species)		
Yarrowia lipolytica	21	2,01
Other fungi (4 species)		
Schizosaccharomyces pombe	13	2,43
Ustilago maydis	16	2,44
Cryptococcus neoformans	16	2,80
Coprinopsis cinerea	16	3,18
Other eukaryotes (8 species)		
Homo sapiens	16	2,58
Caenorhabditis elegans	16	2,71
Caenorhabditis briggsae	16	2,92
Danio rerio	16	3,43
Xenopus laavis	16	3,69
Mus musculus	16	3,80
Drosophila melanogaster	16	4,03
Arabidopsis thaliana	16	4,14

* - D-S - number of residues between conserved Asp of HPD motif and conserved Ser of helix III
# - Distance – evolutionary protein distance from $Jac1_{Sc}$ (details in “Suppl. Methods”)