

## **Supplemental Information**

### **Quantitative Structural Analysis**

#### **of Importin- $\beta$ Flexibility: Paradigm**

#### **for Solenoid Protein Structures**

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#### **Figure S1. Residues targeted in mutagenesis studies; relates to Figure 1**

(A) Close-up views of residues targeted in mutagenesis studies. The structure of unliganded  $\gamma$ Imp $\beta$  is shown in ribbon representation (grey), with the residues targeted in mutagenesis studies in stick representation and colored: red, Ser74 (HEAT repeat H2)/Glu737 (H17); blue, Asp167 (H4)/Asn738 (H17); green, Glu341 (H8)/Phe514 (H12) and Asp343 (H8)/Lys468 (H11). The structure is shown in the analogous orientation as in Figure 1.

(B) Alignment of human, mouse and *S. cerevisiae* (yeast) Imp $\beta$  amino acid sequences. Highlighted are *S. cerevisiae* Imp $\beta$  residues involved in the principal sequence-distal contacts between HEAT repeats H2/H4 and H17 (Ser74 [red], Asp167 [blue], Arg696 [red], Glu737 [red], Asn738 [blue], Gly739 [blue]), and between H8 and H11/H12 (Pro340, Glu341, Asp343, His466, Lys468, Phe514 [green]). The analogous residues in human and mouse Imp $\beta$  are highlighted in the same colours if conserved, and with grey background if not conserved. The sequences were aligned using ClustalW (Larkin et al., 2007).

Figure S1A

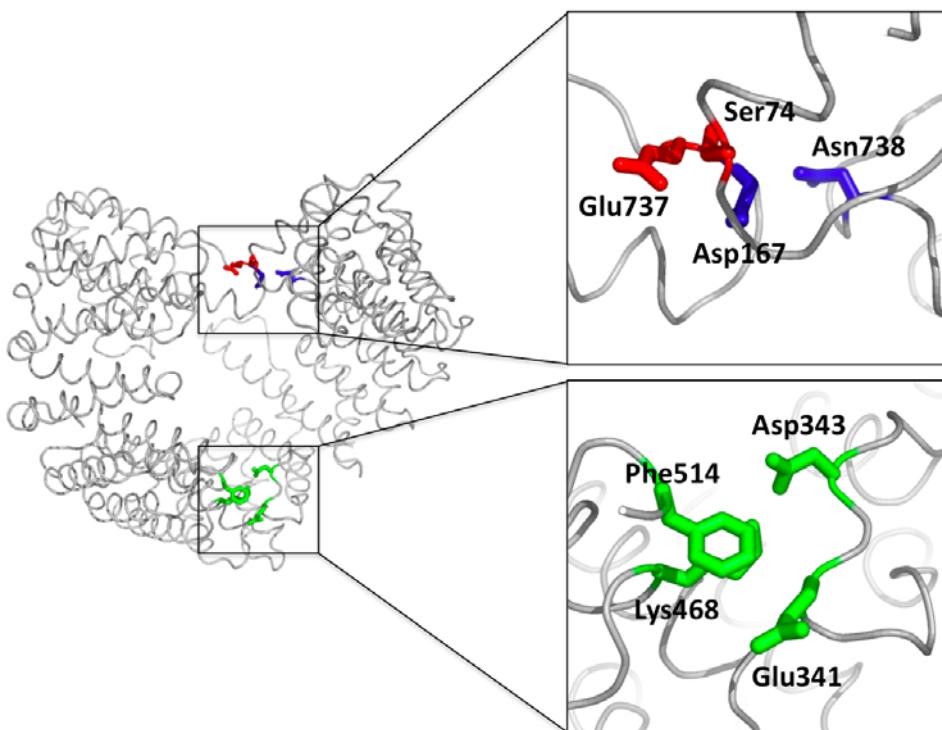


Figure S1B

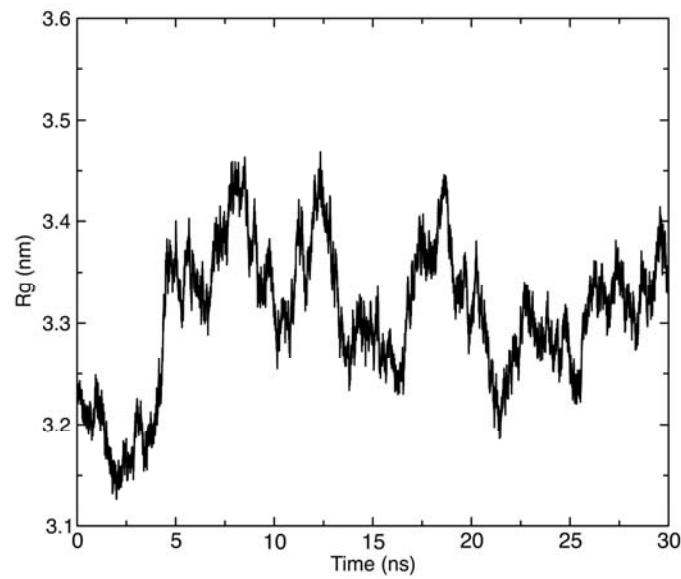
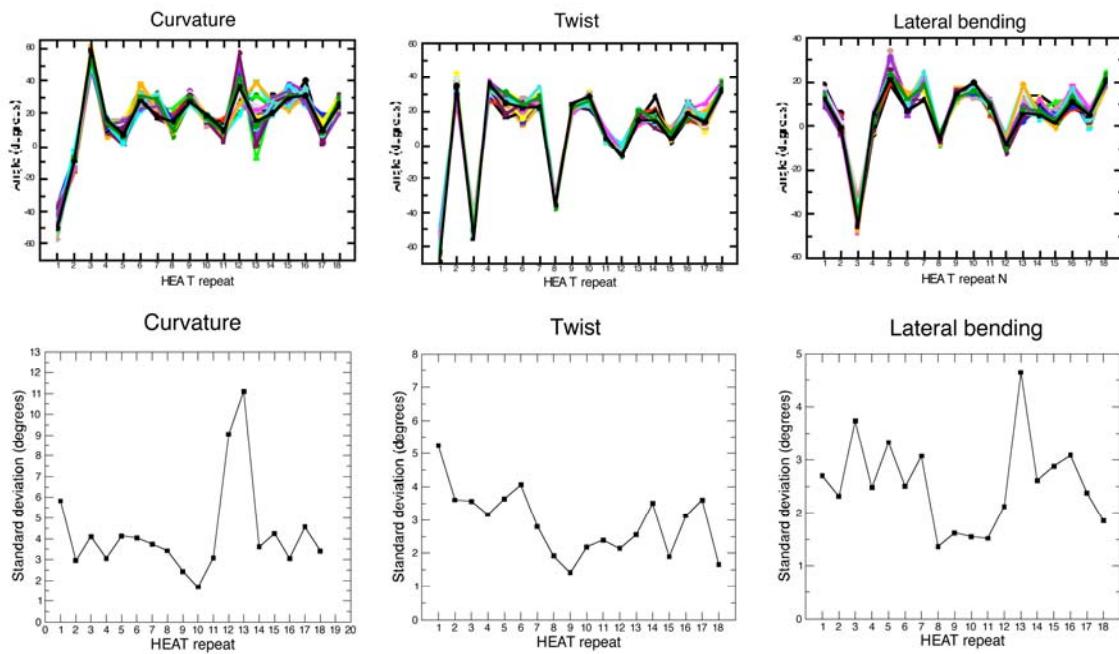
Human	---	MELITILEKTVS - PDRLELEAAQKFLERAAVENLPTFLVELSRVLAN	46
Mouse	---	MELITILEKTVS - PDRLELEAAQKFLERAAVENLPTFLVELSRVLAN	46
Yeast		MSTAEEFAQLLENSILSPDQNIRLTSETQLKKLSNDNFLQFAGLSSQVLID	50
Human	PGNSQVARVAAGLQIKNSLTSKDPDIKAQYQQRWL - AIDANARREVKNYV	95	
Mouse	PGNSQVARVAAGLQIKNSLTSKDPDIKAQYQQRWL - AIDANARREVKNYV	95	
Yeast	ENTKLEGRLAALTALKNELVSKD <span style="background-color: red;">S</span> VKTQQFAQRWITQVSPEAKNQIKTNA	100	
Human	LHTLG - TETYRPSSASQCVGAIACAEIPVNQWPPELI PQLVANVTNPNSTE	144	
Mouse	LQTLG - TETYRPSSASQCVGAIACAEIPVSQWPPELI PQLVANVTNPNSTE	144	
Yeast	LTALVSIEPRIANAAAQLIAAIADIELPHGAWPELMKIMVDNTG - AEQPE	149	
Human	HMKESTLEAIGYICQDID <span style="background-color: blue;">P</span> EQ - LQDKSNEILTAAI <span style="background-color: blue;">I</span> QGMRKEEPSNNVKL	192	
Mouse	HMKESTLEAIGYICQDID <span style="background-color: blue;">P</span> EQ - LQDKSNEILTAAI <span style="background-color: blue;">I</span> QGMRKEEPSNNVKL	192	
Yeast	NVKRASLLALGYMCESAD <span style="background-color: blue;">P</span> QSQALVSSNNILIAIVQGAQSTETSKAVRL	199	
Human	AATNALLNSLEFTKANFDKESERHFIMQVVCEATQCPDTRVRVAALQNLV	242	
Mouse	AATNALLNSLEFTKANFDKESERHFIMQVVCEATQCPDTRVRVAALQNLV	242	
Yeast	AALNALADSLIFIKNNMEREGERNYLMQVVCEATQAEDIEVQAAFGCLC	249	
Human	KIMSLYYQYMETYMGPA <span style="background-color: green;">L</span> FAITIEAMKSDIDEVALQGIEFWNSVCDEEMD	292	
Mouse	KIMSLYYQYMETYMGPA <span style="background-color: green;">L</span> FAITIEAMKSDIDEVALQGIEFWNSVCDEEMD	292	
Yeast	KIMSKYYTFMKPYMEQALYALTIA <span style="background-color: green;">T</span> MKSPNDKVASMTVEFWSTICEEEID	299	
Human	LAIEASEAAEQGRP <span style="background-color: green;">P</span> PEHTSKFYAKGALQYL <span style="background-color: green;">P</span> ILTQTL <span style="background-color: green;">T</span> Q <span style="background-color: green;">L</span> KQDEN <span style="background-color: green;">D</span> DW	342	
Mouse	LAIEASEAAEQGRP <span style="background-color: green;">P</span> PEHTSKFYAKGALQYL <span style="background-color: green;">P</span> ILTQTL <span style="background-color: green;">T</span> Q <span style="background-color: green;">L</span> KQDEN <span style="background-color: green;">D</span> DW	342	
Yeast	IAYELAQFPQ --- SPLQSYNFALSSI <span style="background-color: green;">K</span> DVVPNLLNLLTRQNED <span style="background-color: green;">P</span> E <span style="background-color: green;">D</span> DW	345	
Human	NPCKAAGVCLMLLATCCEDDIVPHVLPFIKEHI <span style="background-color: green;">K</span> NPDWRYRDAAVMAFGC	392	
Mouse	NPCKAAGVCLMLLSTCCEDDIVPHVLPFIKEHI <span style="background-color: green;">K</span> NPDWRYRDAAVMAFGS	392	
Yeast	NVSMSAGACLQLFAQNCGNHILEPVLEFVEQNITADNWRNREAAVMAFGS	395	
Human	ILEGPEPSQLKPLVIQAMPTLIELMKDPSVVVRDTAAWTVGRICELLPEA	442	
Mouse	ILEGPEPNQLKPLVIQAMPTLIELMKDPSVVVRDTAAWTVGRICELLPEA	442	
Yeast	IMDGPDKVQRTYYVHQALPSILNLMNDQLQVKETTAWCIGRIADSVAES	445	
Human	AINDVYLAPLLQCLIEGLSA <span style="background-color: green;">E</span> P <span style="background-color: green;">R</span> VASNV <span style="background-color: green;">C</span> WAFSSLAEAA <span style="background-color: green;">Y</span> EAADVADDQE	492	
Mouse	AINDVYLAPLLQCLIEGLSA <span style="background-color: green;">E</span> P <span style="background-color: green;">R</span> VASNV <span style="background-color: green;">C</span> WAFSSLAEAA <span style="background-color: green;">Y</span> EAADVADDQE	492	
Yeast	IDPQQHLPGVVQACLIGLQD <span style="background-color: green;">T</span> <span style="background-color: green;">P</span> VATNC <span style="background-color: green;">S</span> WTIINLVEQLAEATPSP ---	491	
Human	EPATYCLSSSFELIVQKL <span style="background-color: green;">L</span> LETTDRPDGH <span style="background-color: green;">Q</span> ONNL <span style="background-color: green;">R</span> SAYESLMEIV <span style="background-color: green;">V</span> KNSAKD	542	
Mouse	EPATYCLSSSFELIVQKL <span style="background-color: green;">L</span> LETTDRPDGH <span style="background-color: green;">Q</span> ONNL <span style="background-color: green;">R</span> SAYESLMEIV <span style="background-color: green;">V</span> KNSAKD	542	
Yeast	-----I <span style="background-color: green;">Y</span> NFYPALVDGLIGAANRIDNE <span style="background-color: green;">P</span> N-ARASAFSALT <span style="background-color: green;">T</span> MVEYATDT	534	
Human	CYPAVQKTTLVIMERLQQVLQMESH <span style="background-color: green;">I</span> QSTS <span style="background-color: green;">D</span> R <span style="background-color: green;">I</span> QFNDL <span style="background-color: green;">Q</span> SLCATL <span style="background-color: green;">Q</span> NVL	592	
Mouse	CYPAVQKTTLVIMERLQQVLQMESH <span style="background-color: green;">I</span> QSTS <span style="background-color: green;">D</span> R <span style="background-color: green;">I</span> QFNDL <span style="background-color: green;">Q</span> SLCATL <span style="background-color: green;">Q</span> NVL	592	
Yeast	VAETSASISTFVMDKLGQTMSVDENQLTLEDAQSLQELQSNI <span style="background-color: green;">L</span> TVLAAVI	584	
Human	RKVHQHDALQISDV <span style="background-color: green;">V</span> MASLLRMFQ <span style="background-color: green;">S</span> TAGSGGV <span style="background-color: green;">Q</span> EDALMAVST <span style="background-color: green;">T</span> LV <span style="background-color: green;">E</span> VL <span style="background-color: green;">G</span> GE	642	
Mouse	RKVHQHDALQISDV <span style="background-color: green;">V</span> MASLLRMFQ <span style="background-color: green;">S</span> TAGSGGV <span style="background-color: green;">Q</span> EDALMAVST <span style="background-color: green;">T</span> LV <span style="background-color: green;">E</span> VL <span style="background-color: green;">G</span> GE	642	
Yeast	RKSPSS - VEPVADMLMGLFFR <span style="background-color: green;">I</span> LEKK <span style="background-color: green;">D</span> -SAFIEDDV <span style="background-color: green;">F</span> YAI <span style="background-color: green;">S</span> ALAASL <span style="background-color: green;">G</span> K <span style="background-color: green;">K</span>	632	
Human	FLKYMEAFKP <span style="background-color: green;">F</span> LGIGLK <span style="background-color: green;">N</span> YA <span style="background-color: green;">E</span> YQV <span style="background-color: green;">C</span> LA <span style="background-color: green;">V</span> GL <span style="background-color: green;">G</span> DLC <span style="background-color: green;">R</span> AL <span style="background-color: green;">Q</span> SN <span style="background-color: green;">I</span> IPFC <span style="background-color: green;">D</span> EV	692	
Mouse	FLKYMEAFKP <span style="background-color: green;">F</span> LGIGLK <span style="background-color: green;">N</span> YA <span style="background-color: green;">E</span> YQV <span style="background-color: green;">C</span> LA <span style="background-color: green;">V</span> GL <span style="background-color: green;">G</span> DLC <span style="background-color: green;">R</span> AL <span style="background-color: green;">Q</span> SN <span style="background-color: green;">I</span> LPFC <span style="background-color: green;">D</span> EV	692	
Yeast	FEKYLETFSPYLLKALN-QVDS <span style="background-color: green;">P</span> V <span style="background-color: green;">S</span> ITAV <span style="background-color: green;">G</span> FI <span style="background-color: green;">A</span> DISNSLEEDFRRYSDAM	681	
Human	MQLLLENLGNENVH <span style="background-color: red;">R</span> SVKP <span style="background-color: red;">Q</span> ILSV <span style="background-color: red;">F</span> G <span style="background-color: red;">D</span> IALAIG <span style="background-color: red;">G</span> EF <span style="background-color: red;">K</span> KK <span style="background-color: red;">Y</span> LEV <span style="background-color: red;">V</span> LN <span style="background-color: red;">T</span> Q <span style="background-color: red;">Q</span> A	742	
Mouse	MQLLLENLGNENVH <span style="background-color: red;">R</span> SVKP <span style="background-color: red;">Q</span> ILSV <span style="background-color: red;">F</span> G <span style="background-color: red;">D</span> IALAIG <span style="background-color: red;">G</span> EF <span style="background-color: red;">K</span> KK <span style="background-color: red;">Y</span> LEV <span style="background-color: red;">V</span> LN <span style="background-color: red;">T</span> Q <span style="background-color: red;">Q</span> A	742	
Yeast	MNVLAQMI <span style="background-color: red;">S</span> PN <span style="background-color: red;">N</span> AR <span style="background-color: red;">R</span> ELKPAV <span style="background-color: red;">L</span> SV <span style="background-color: red;">F</span> G <span style="background-color: red;">D</span> IASN <span style="background-color: red;">I</span> GAD <span style="background-color: red;">F</span> I <span style="background-color: red;">P</span> Y <span style="background-color: red;">L</span> ND <span style="background-color: red;">I</span> MALCVAA	731	
Human	SQAQV <span style="background-color: blue;">D</span> K <span style="background-color: blue;">K</span> SDYDMV <span style="background-color: blue;">D</span> YLNELRES <span style="background-color: blue;">C</span> LEAYTG <span style="background-color: blue;">I</span> V <span style="background-color: blue;">Q</span> GL <span style="background-color: blue;">K</span> GD <span style="background-color: blue;">Q</span> ENV <span style="background-color: blue;">V</span> PD <span style="background-color: blue;">V</span> ML <span style="background-color: blue;">V</span> QP	792	
Mouse	SQAQV <span style="background-color: blue;">D</span> K <span style="background-color: blue;">K</span> SDFDMV <span style="background-color: blue;">D</span> YLNELRES <span style="background-color: blue;">C</span> LEAYTG <span style="background-color: blue;">I</span> V <span style="background-color: blue;">Q</span> GL <span style="background-color: blue;">K</span> GD <span style="background-color: blue;">Q</span> ENV <span style="background-color: blue;">V</span> PD <span style="background-color: blue;">V</span> ML <span style="background-color: blue;">V</span> QP	792	

Yeast	QNTKPE <b>ENG</b> TLEALDYQIKVLEAVLDAYVGIVAGLHDKPE-----ALFP	774
Human	RVEFILSFIDHIAGDE--DHTDGVVACAAGLIGDLCTAFGKDVLKLVEAR	840
Mouse	RVEFILSFIDHIAGDE--DHTDGVVACAAGLIGDLCTAFGKDVLKLVEAR	840
Yeast	YVGTIFQFIAQVAEDPQLYSEDATSRAAVGLIGDIAAMFPDGSIKQFYGQ	824
Human	PMIHELLTEGRRS--KTNKAKTLARWATKELRKLKNQA	876
Mouse	PMIHELLTEGRRS--KTNKAKTLATWATKELRKLKNQA	876
Yeast	DWVIDYIKRTRSGQLFSQATKDTARWAREQQKRQLSL-	861

**Figure S2. Additional molecular dynamics simulations of yImp $\beta$  and variation of inter-repeat angles along the polypeptide chain for simulated structures; relates to Figure 2**

(A) Evolution of radius of gyration of during an additional molecular dynamics simulation of yImp $\beta$ . The starting condition of the simulation was the crystal structure. The behaviour of the protein during the simulation resembles the behaviour during the original simulation (Figure 3), but switches more frequently between the extended and compact conformations.

(B) Variation of inter-repeat angles along the polypeptide chain for simulated structures. Top row: curvature (left), twist (middle) and lateral bending angles (right panels) for the original state and 30 structures after each ns along the molecular dynamics trajectory shown in (A). Bottom row: standard deviations for curvature, twist and lateral bending angle values.

**A****B**

**Table S1. Yeast strains and plasmids used in this study; relates to Figure 4**

Strain/Plasmid	Description	Origin
FY23 (ACY192)	Wild type, <i>MATa ura3-52 leu2ΔI trp1</i>	(Winston et al., 1995)
PSY884 (ACY209)	<i>ΔRSL1::HIS3 [RSL1 CEN URA3 AMP<sup>R</sup>], MATa ade2 leu2 trp1 ura3</i>	(Koepf et al., 1996)
ACY639	<i>srp1-31, MATα trp- ura- leu- lys- his-</i>	(Loeb et al., 1995)
pRS315 (pAC3)	<i>CEN LEU2 AMP<sup>R</sup></i>	(Sikorski and Hieter, 1989)
pAC62	<i>RSL1, CEN LEU2 AMP<sup>R</sup></i>	This Study
pAC511	<i>RSL1-GFP, CEN TRP1 AMP<sup>R</sup></i>	This Study
pAC1065	<i>SV40TAgNLS-GFP-GFP, CEN URA3 AMP<sup>R</sup></i>	(Hodel et al., 2006)
pAC1069	<i>GFP-GFP, CEN URA3 AMP<sup>R</sup></i>	(Hodel et al., 2006)
pAC2460	<i>S74K RSL1-GFP, CEN TRP1 AMP<sup>R</sup></i>	This Study
pAC2461	<i>D167K RSL1-GFP, CEN TRP1 AMP<sup>R</sup></i>	This Study
pAC2462	<i>E341A RSL1-GFP, CEN TRP1 AMP<sup>R</sup></i>	This Study
pAC2463	<i>K468D RSL1-GFP, CEN TRP1 AMP<sup>R</sup></i>	This Study
pAC2464	<i>F514K RSL1-GFP, CEN TRP1 AMP<sup>R</sup></i>	This Study
pAC2465	<i>E737K RSL1-GFP, CEN TRP1 AMP<sup>R</sup></i>	This Study
pAC2466	<i>S74K RSL1, CEN LEU2 AMP<sup>R</sup></i>	This Study
pAC2467	<i>D167K RSL1, CEN LEU2 AMP<sup>R</sup></i>	This Study
pAC2468	<i>E341A RSL1, CEN LEU2 AMP<sup>R</sup></i>	This Study
pAC2469	<i>K468D RSL1, CEN LEU2 AMP<sup>R</sup></i>	This Study
pAC2470	<i>F514K RSL1, CEN LEU2 AMP<sup>R</sup></i>	This Study
pAC2471	<i>E737K RSL1, CEN LEU2 AMP<sup>R</sup></i>	This Study
pAC2536	<i>S74K/F514K RSL1-GFP, CEN TRP1 AMP<sup>R</sup></i>	This Study
pAC2537	<i>S74K/F514K RSL1, CEN LEU2 AMP<sup>R</sup></i>	This Study

## SUPPLEMENTAL REFERENCES

- Hodel, A.E., Harreman, M.T., Pulliam, K.F., Harben, M.E., Holmes, J.S., Hodel, M.R., Berland, K.M., and Corbett, A.H. (2006). Nuclear localization signal receptor affinity correlates with in vivo localization in *Saccharomyces cerevisiae*. *J. Biol. Chem.* *281*, 23545-23556.
- Koepp, D.M., Wong, D.H., Corbett, A.H., and Silver, P.A. (1996). Dynamic localization of the nuclear import receptor and its interactions with transport factors. *J. Cell Biol.* *133*, 1163-1176.
- Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., *et al.* (2007). Clustal W and Clustal X version 2.0. *Bioinformatics* *23*, 2947-2948.
- Loeb, J.D.J., Schlenstedt, G., Pellman, D., Kornitzer, D., Silver, P.A., and Fink, G.R. (1995). The yeast nuclear import receptor is required for mitosis. *Proc. Natl. Acad. Sci. USA* *92*, 7647-7651.
- Sikorski, R.S., and Hieter, P. (1989). A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. *Genetics* *122*, 19-27.
- Winston, F., Dollard, C., and Ricupero-Hovasse, S.L. (1995). Construction of a set of convenient *Saccharomyces cerevisiae* strains that are isogenic to S288C. *Yeast* *11*, 53-55.