Supporting Information

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SI Text

Yeast Strains. CAY1049 (*MAT**a** his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 sse1Δ100*::*hphMX4 sse2*Δ::*kanMX4* [pCUA-SSE1]) was constructed in several steps. First, the complete *SSE1* locus including 540 bp of the promoter and 186 bp of the terminator was deleted by transforming BY4742 (1) with an *hphMX4* deletion cassette amplified from pAG32 (2) using primers GAAGCCTTTTTGTTCCATAGTAGTTGTTTACTACGCGTGTATATCTTCCAGCTGAAGCTTCGTACGC and CATATATTTCACTTAGAGGGTTTAGGAGACTAATGCGTGAATACGCGCTGGCGCATAGGCCACTAGTGGATCTG. Deletion was verified by PCR analysis. The resulting strain was transformed with pCUA-SSE1 (3) to uracil prototrophy and mated with a *sse2*Δ::*kanMX4* derivative of BY4741 (Euroscarf accession number Y07167), and CAY1049 was recovered as a meotic segregant. In the cross, all heterozygous markers exhibited 2:2 segregation, and the *sse1* slow growth phenotype always comigrated with the deletion marker.

CAY1134 (*MAT**a** his3Δ1 leu2Δ0 ura3Δ0 met15Δ0 sse1Δ100*::*hphMX4 sse2*Δ::*kanMX4 fes1*Δ::*LEU2* [pCUA-SSE1]) was constructed by transforming CAY1049 with a PCR-amplified *LEU2* cassette flanked by homology upstream and downstream of *FES1* (primers: CACATACATAACTATGGAAAAGCTATTACAGTGGTCTATTGCGAATTCTCAGAAATATCTTGACCGCAG and CTACGGACGTAATCATAATACATACTTTACGGCTAAATAATCGTCCTACCCTATGAACATATTCC). Correct integration in the *FES1* locus was verified by PCR. The *LEU2* marker segregated 2:2 in crosses.

The complete locus of *SSE1* including the endogenous promoter and terminator was introduced together with *CEN4*/*ARS1* sequences into the *HIS3* vector pRS303 (4) creating pCA503. C-terminal truncation alleles of *SSE1* were obtained by introducing deletions in pCA503 using single-stranded mutagenesis. The resulting plasmids were introduced in CAY1049 and CAY1134 by transformation. HIS^+ and URA^- derivatives of the transformants were selected on 5-FOA medium.

Supporting information (SI) Tables S1–S6: Hydrogen–Deuterium Exchange Data. Tables S1–S6 contain the average number of deuterons incorporated in segments of Sse1 or Ssa1 after H/D exchange for the various conditions and protein complexes studied. In most cases, two datasets from independent experiments are presented. "Start" and "End" indicate the boundaries of the respective peptides. These peptides were identified by their MS/MS spectra or their exact mass. Note that the two N-terminal amide positions of each peptide undergo fast back-exchange, resulting in the loss of HX information for these positions. Moreover, slight differences occur between the datasets because of to different back-exchange in distinct experiments. 100% represents the experimental maximum deuteration as obtained from measurement of a protein sample denatured in 6 M urea in D_2O buffer. N.D. indicates lack of data because of peptide overlap or insufficient signal intensity. Difference plots obtained from pairwise comparison of the numbers of deuterons incorporated under different conditions are presented in Figs. S1, S2, S5, S6 and S8. For Table S1, the corresponding difference plots are shown in Fig. S1. For Tables S2 and S3, the corresponding difference plots are shown in Fig. S2. For Table S4, the corresponding difference plot is shown in Fig. S6*A*. For Table S5, the corresponding difference plots are shown in Fig. S5. For Table S6, the mass spectra of representative peptides are shown in Fig. S7, and difference plots are shown in Fig. S8.

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2. Goldstein AL, McCusker JH (1999) Three new dominant drug resistance cassettes for gene disruption in *Saccharomyces cerevisiae*. *Yeast* 15:1541–1553.
3. Raviol H, Sadlish H, Rodriguez F, Mayer MP, Bukau B (2006) Chaperone network in the yeast cytosol: Hsp110 is revealed as an Hsp70 nucleotide exchange factor. *EMBO J* 25:2510–2518.
4. Sikorski RS, 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.
5. Andréasson C, Fiaux J, Rampelt H, Mayer MP, Bukau B (2008) Hsp110 is a nucleotide-activated exchange factor for Hsp70. *J Biol Chem* 283:8877–8884.
6. Theyssen H, Schuster HP, Packschies L, Bukau B, Reinstein J (1996) The second step of ATP binding to DnaK induces peptide release. *J Mol Biol* 263:657–670.
7. Sadlish H, *et al*. (2008) Hsp110 chaperones regulate prion formation and propagation in *S. cerevisiae* by two discrete activities. *PloS ONE* 3:e1763.
8. Flaherty KM, DeLuca-Flaherty C, McKay DB (1990) Three-dimensional structure of the ATPase fragment of a 70 K heat-shock cognate protein. *Nature* 346:623–628.

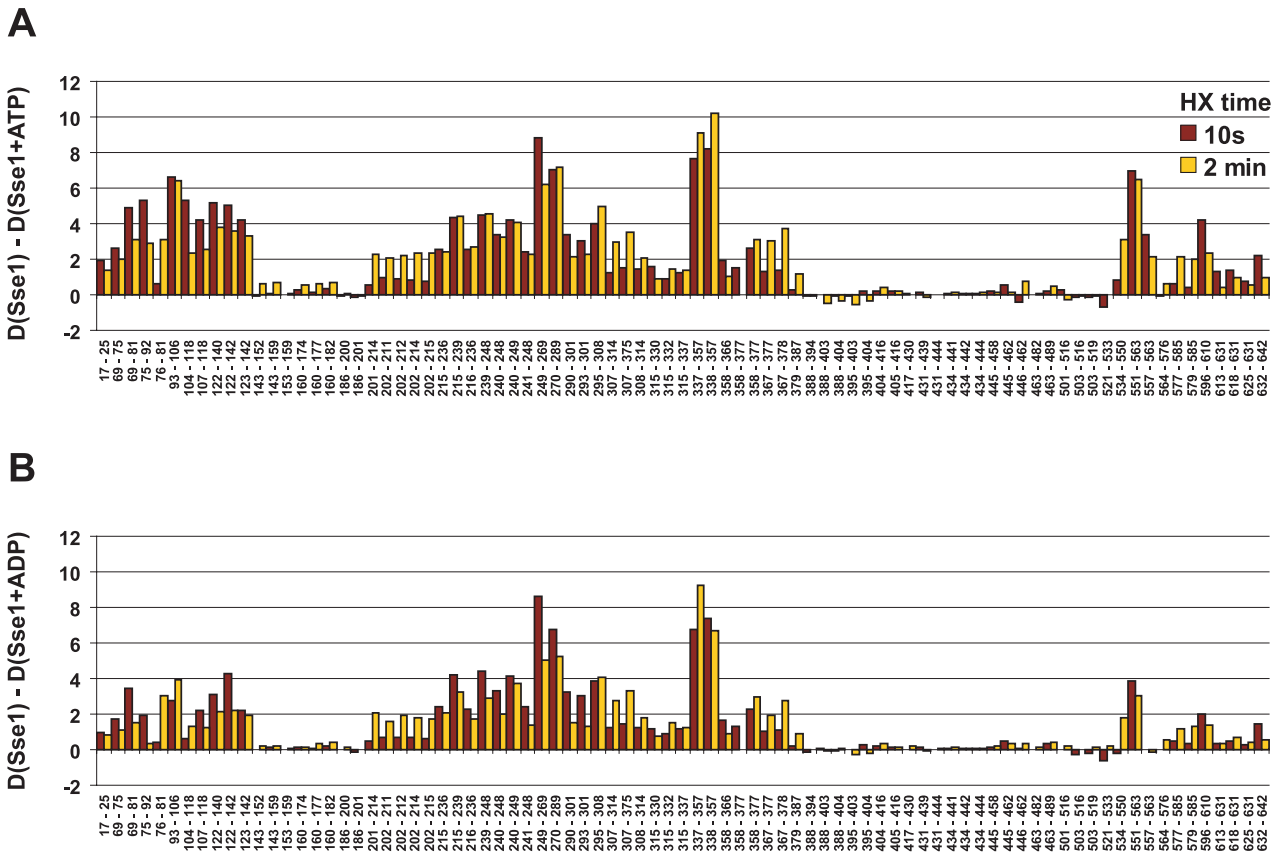

Fig. S1. HX protection in Sse1 induced by nucleotide binding. For each Sse1 peptide identified, the difference in deuteron incorporation between samples of Sse1 nucleotide-free and Sse1 in the presence of ATP (*A*) or ATP-free ADP (*B*) is plotted for HX reaction times 10 s and 2 min. The most complete dataset of Table S1 (Dataset 1) is shown. The individual peptides are indicated by the start and end residue numbers of the corresponding segments.

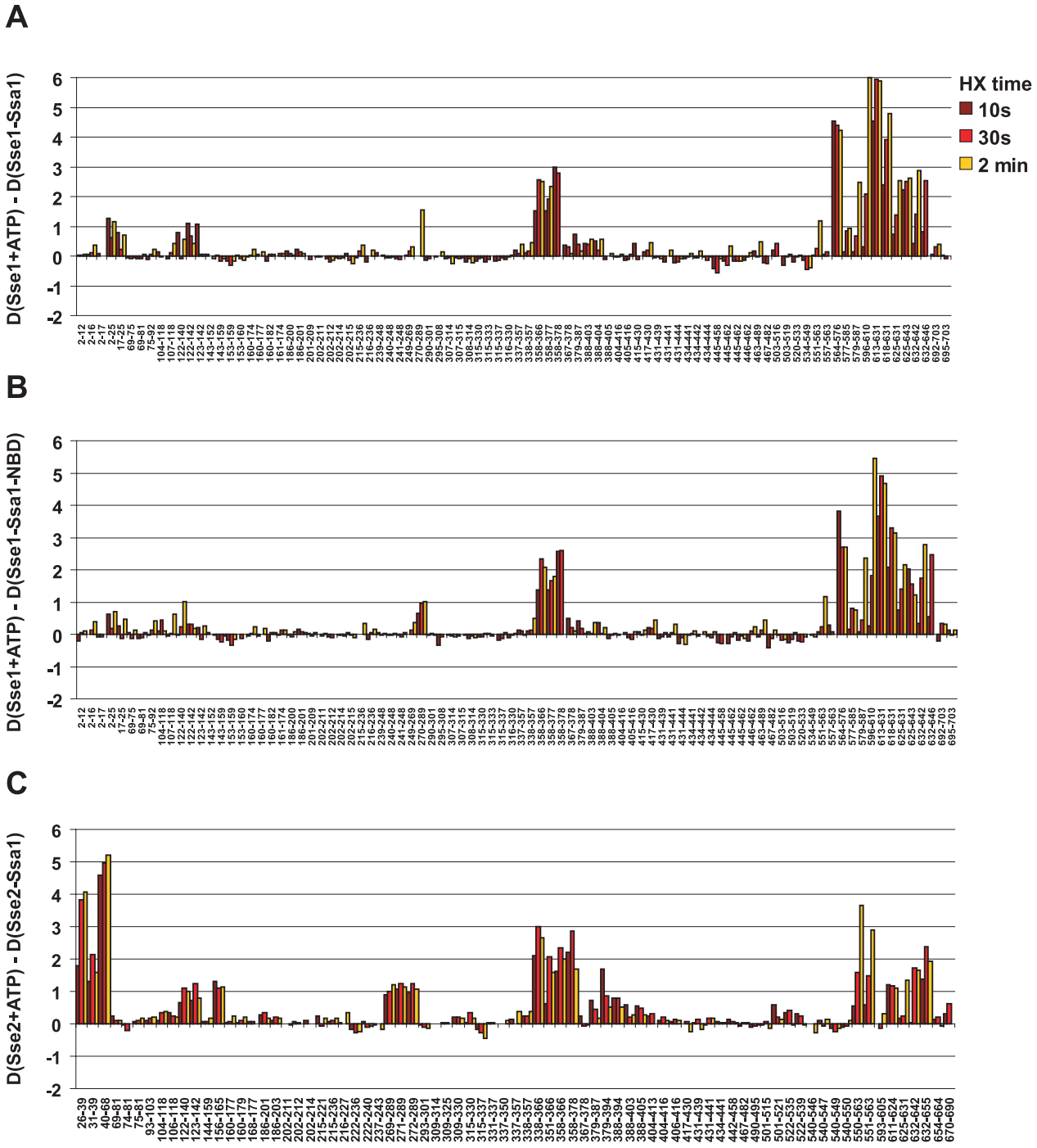

Fig. S2. HX protection induced in Sse1 and Sse2 by Ssa1 binding. For each Sse1 or Sse2 peptide identified, the difference in deuteron incorporation between samples of Sse1 + ATP (*A* and *B*) or Sse2 + ATP (*C*) alone and of their nucleotide-free complex with Ssa1 (*A* and *C*) or Ssa1-NBD (*B*) is plotted for several HX reaction times. The data from Table S3 and from the most complete dataset of Table S2 (Dataset 1) are shown. To avoid effects originating from ATP release from Sse1/2, only the data for short HX times are displayed (10 s–2 min). Note that the peptides are slightly different for *A*, *B*, and for *C* because they are obtained from different protein isoforms. Peptides harboring a minimum difference in deuteron incorporation of 0.4 Da and 5% of the total possible exchange are mapped as protected in Fig. 1*D*.

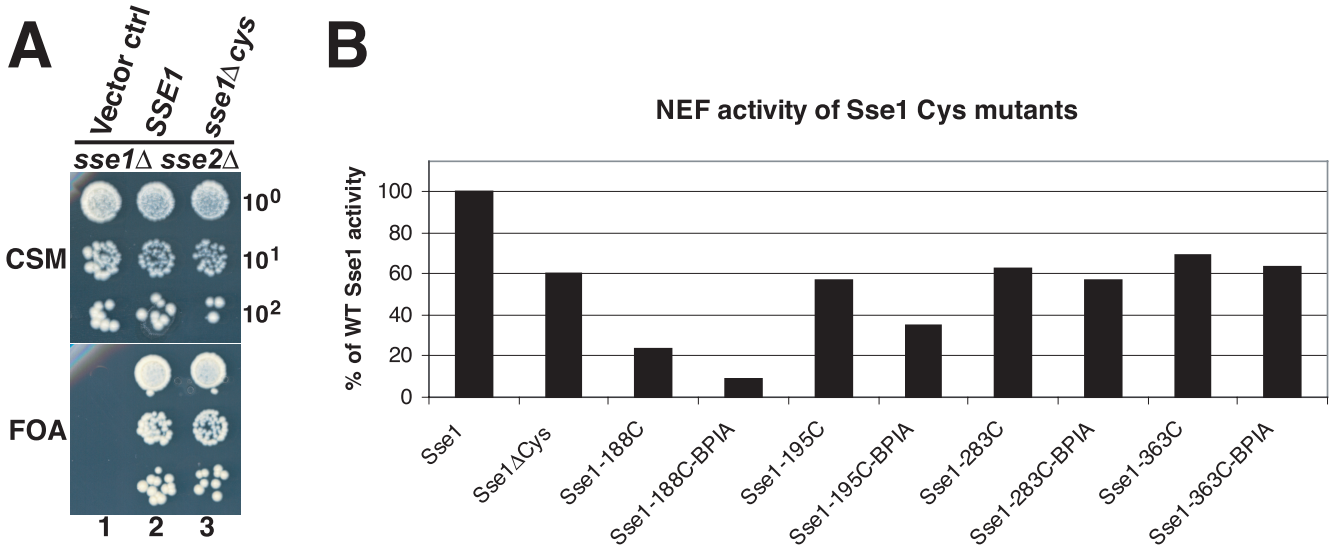

Fig. S3. The cysteine-free protein Sse1ΔCys is functional. The five endogenous cysteines of Sse1 were replaced with residues found at the corresponding positions in fungal Hsp110 homologs (C142S, C211V, C228Y, C380S, and C484L), and the resulting allele *SSE1ΔCys* was introduced in a vector for Sse1 expression in yeast and *Escherichia coli*. (*A*) Ten-fold serial dilutions of *sse1Δ sse2Δ* yeast strains carrying *SSE1* or *SSE1ΔCys* in the context of its endogenous locus on a single copy plasmid were spotted onto complete synthetic medium (CSM) or 5-fluoroorotic acid (FOA) medium and incubated at 30°C for 2 days. (*B*) Relative NEF activity of Sse1 or derived cysteine mutants, with or without BPIA labeling. The Sse1 protein variants were preincubated with 3 mM ATP before rapid mixing with Ssa1 (0.5 μM) each and measuring of the MABA-ADP release rates with a stopped-flow instrument (5, 6).

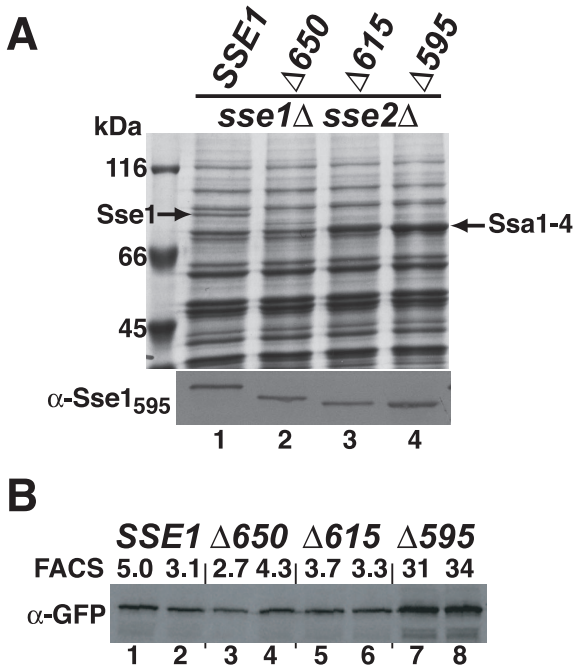

Fig. S4. Cells adapt to deficiencies in Sse1 activity by up-regulating the expression of Ssa3/4. (*A*) SDS/PAGE and Western blot analysis (α-Sse1$_{595}$) of total protein lysates from *sse1*Δ *sse2*Δ yeast strains used in Fig. 3*B*. Coomassie brilliant blue-stained bands corresponding to Sse1 and Ssa are marked. (*B*) A heat shock-responsive P_{SSA4}-yEGFP reporter was integrated into the chromosome of the strains used in *A*, and expression of yEGFP of duplicate clones was assessed using fluorescence-activated cell sorting (FACS, mean value indicated) and Western blot analysis (α-GFP). Presumably, cells with impaired Sse1/2 NEF activity suffer from insufficient levels of ATP-bound Hsp70. Increasing the total Hsp70 expression level might partially alleviate the condition and allow growth by increasing the amount of available ATP-bound Ssa1–4. A similar mitigation of an *sse1*Δ phenotype by Ssa1 overproduction has been reported for the propagation of the yeast prion [PSI$^+$] (10).

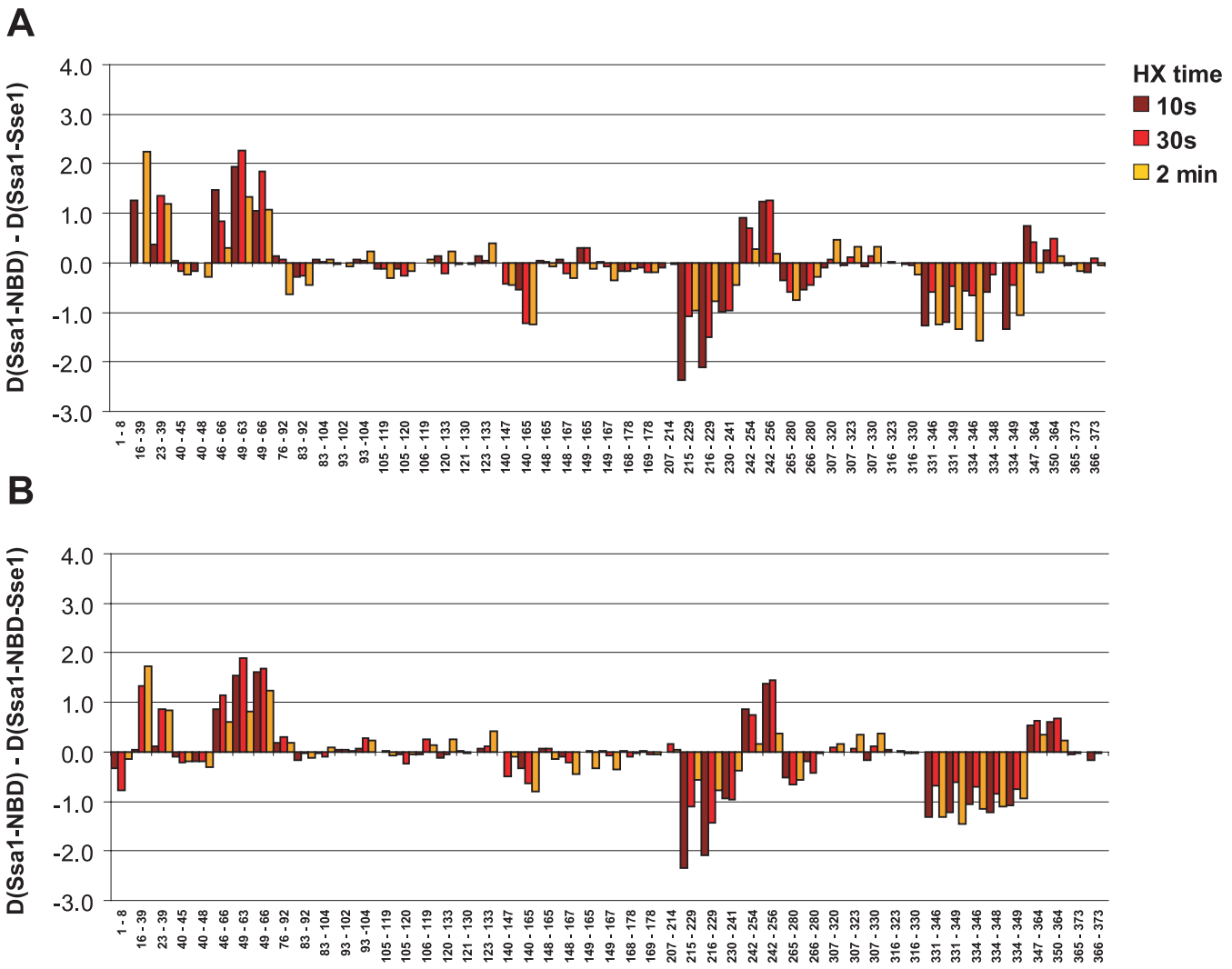

Fig. S5. HX protection and deprotection induced in Ssa1-NBD by Sse1 binding. For each Ssa1-NBD peptide identified, the difference in deuteron incorporation between samples of nucleotide-free Ssa1-NBD and of nucleotide-free complex of Ssa1-NBD (*A*) or Ssa1 (*B*) with Sse1 is plotted for several HX reaction times. The most complete dataset of Table S5 (Dataset 1) is shown. Note that because of stochastic aggregation problems during the 2-h HX reaction, the data for this time point are not considered. Peptides harboring a minimum absolute difference in deuteron incorporation of 0.6 Da and 5% of the total possible exchange are mapped as protected, respectively, deprotected in Fig. 4*A*.

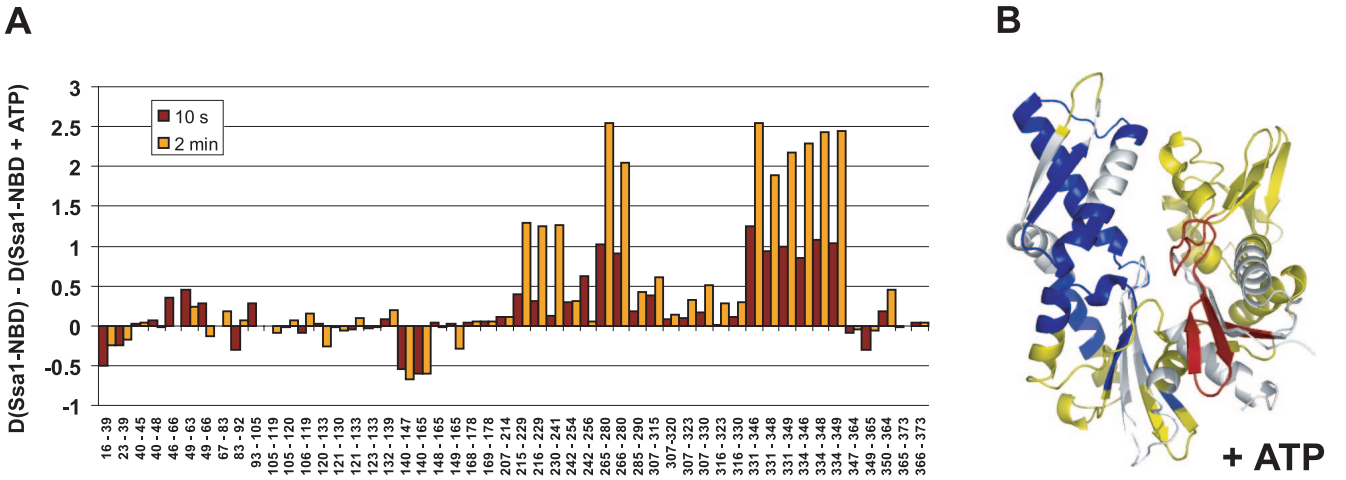

Fig. S6. ATP stabilizes lobe II of Ssa1-NBD. (*A*) Difference in deuteron incorporation between nucleotide-free Ssa1-NBD and Ssa1-NBD in the presence of 1 mM ATP after 10-s or 2-min HX (data of Table S4). The individual peptides are indicated by the start and end residue numbers of the corresponding segments. (*B*) The Ssa1-NBD modeled onto the structure of the Hsc70 NBD (8), as depicted in Figs. 4*A* and 5*A*. The molecule is colored according to the observed pattern of ATP-induced HX protection (blue) and deprotection (red). Yellow indicates no change in HX properties, and gray, no data available.

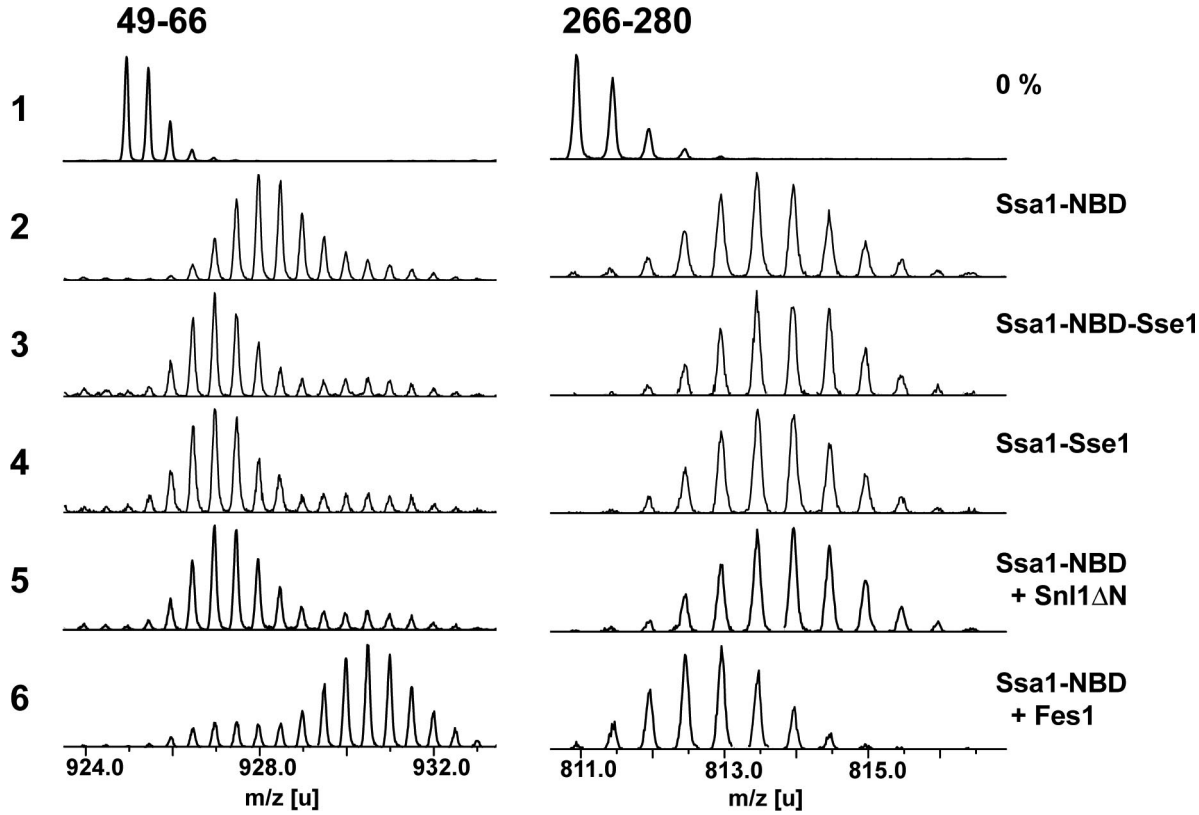

Fig. S7. Effect of Sse1, Snl1-ΔN, and Fes1 on the NBD of Ssa1. Mass spectra of representative peptides of the NBD after 10-s hydrogen-deuterium exchange. Peptide 49–66 belongs to lobe IB, and peptide 266–280 belongs to lobe IIB of the NBD. The protein samples consisted of a final volume of 100 μl of NBD 3.1 μM, no HX (trace 1); Ssa1-NBD 3.1 μM (trace 2); preformed Ssa1-NBD-Sse1-Strep-Tag II complex 2.7 μM (trace 3); preformed Ssa1-His_{10}-Sse1-Strep-Tag II complex 2.7 μM (trace 4); Ssa1-NBD 2.5 μM + Snl1-ΔN 25.0 μM (trace 5); and Ssa1-NBD 2.5 μM + Fes1 7.5 μM (trace 6).

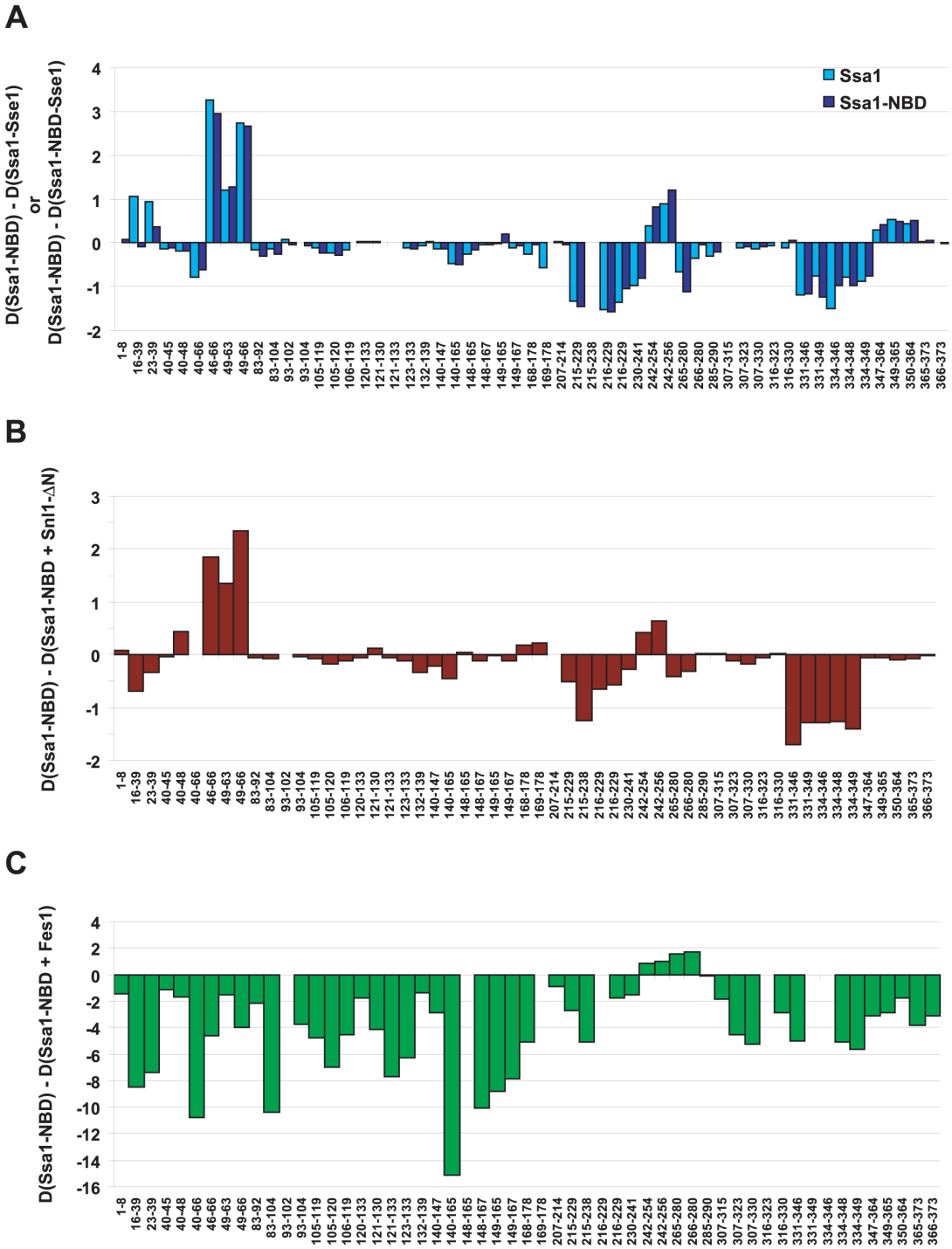

Fig. S8. HX protection and deprotection induced in Ssa1-NBD by binding of the yeast nucleotide exchange factors. For each Ssa1-NBD peptide identified, the difference in deuteron incorporation between samples of nucleotide-free Ssa1-NBD and of nucleotide-free Ssa1-NBD or Ssa1 in the presence of Sse1 (*A*), Snl1-ΔN (*B*), and Fes1 (*C*) is plotted for a 10-s HX reaction. The most complete dataset of Table S6 (Dataset 1) is presented. Peptides harboring a minimum absolute difference in deuteron incorporation of 0.6 Da and 5% of the total possible exchange are mapped as protected, respectively, deprotected in Fig. 4*A*.

Other Supporting Information Files

Table S1 (PDF)
Table S2 (PDF)
Table S3 (PDF)
Table S4 (PDF)
Table S5 (PDF)
Table S6 (PDF)