Supporting Information

Molecular chaperones and stress-inducible protein sorting factors coordinate the spatio-temporal distribution of protein aggregates

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SUPPORTING DATA

We used the NucPred server from the Stockholm Bioinformatics Center to predict nuclear localization sequences in Btn2, Cur1 and Sis1 (Predicted NLS motives are shown in red below). The presence of a predicted NLS and a NucPred score of higher than 0.8 correctly predicts nuclear localization in more than 90% of the cases [1].

Results for Btn2 (NucPred score 0.92):

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MFSIFNSPCVFELPSFSQPLHSRYFDCCSPIVSVYYPECKRRKAIRONLRAPKKS
DANCSEPLRYALEPNGYTLSLSKRIYLFSKYVEKNKELKENHRPTYHV
VQDFGGNQYVEDEADALLRSALKDLDFRAIGKKIADKLFQDYEIELNHGDD
ELSILSKKDKIFKEFSDLQVFEDVFGICGGVENIDDGSREKYALLKIGLVCHEEIS
EGGNEPKMPIIESKIDESHDVNMSESLOKEEEAAEKEPLTQKKKKEEER
LMQEESEKSEQKAKEDEERQKKEEAMEALKARSKELQKQKTSQKKKQ
SKSLPISEIEASNKNNSNSGSAESDNSINSDSDDTVLDFSVSGNTLKKHASPLL
EDVEDEEVRNYESLSRSQKGSNSEEI
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Results for Cur1 (NucPred score 0.85):

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MAAACICQPNLLEINVDGPLDMMIRKKRQIQQPQLRPPLRENKCQPQHFSVRKVN
QSYISSLHKEITCQLIAEVKQKLSRIWEKICVPYELISDKDGNIYVEQSVENDR
LTSEIMEKLDPSNNIDIEAEIIFDDYHLELSRTLNGIISANDHFCYREFSFNNIDDN
```
Results for Sis1 (NucPred score 0.64):

MVKETKLYDLLGVSPSANEQELKKGYRKAALKYHPDKPTGDTEKFKEISEAFEILN
DPOKREIYDQYGLEAARSGGPSFGPGPAGGAGGFPGGAGGFSGGHAFSNEDAFNIFSQFFGSSP
FGADDGSFSFSSYPSGAGMGMPGGMGMHGGMGMPGFGFRSASSSPTYPEEEETVQVNLP
VSLEDLFVGGKKKSFKIGRKPGHGASEKTQIDIQKLPGWKAGTKITYKNQGDYNPDTGRK
LQFVIEKSHPNFKRGDDILYTLPLSFKESLLGFSDKTIQTIDGRTLPLSRVQPVP
QPSQTSTYPQGMPTPKNPSQRGNLIVKYKDVYPSLNDQAQKRAIDENF

SUPPORTING MATERIALS AND METHODS

Plasmids

Table S1 gives an overview of the plasmids that were used in this study.

Table S1: Plasmids used in this study.

| #  | Accession number | Plasmid name                  |
|----|------------------|-------------------------------|
| 1  | O-2167           | pAG415ADH1-Sis1               |
| 2  | O-1986           | pAG416ADH1-Sis1               |
| 3  | O-2150           | pAG415GPD-Sis1                |
| 4  | O-1955           | pAG416GPD-Sis1                |
| 5  | O-2012           | pAG413MET3-Sis1               |
| 6  | O-2011           | pAG416MET3-Sis1               |
| 7  | O-2193           | pAG415ADH1-Sis1-EGFP          |
| 8  | O-1372           | pAG415GPD-Sis1-ΔC-EGFP        |
| 9  | O-2196           | pAG415ADH1-Sis1-ΔC-EGFP       |
| 10 | O-2213           | pAG415GPD-Sis1-ΔC-EGFP        |
| 11 | O-2229           | pAG425GPD-Sis1-ΔC-mCherry     |
| 12 | O-2337           | pAG304GPD-Sis1                |
| 13 | O-2192           | pAG415GPD-Sis1-HA             |
| 14 | O-2092           | pAG413GPD-Sis1-EGFP           |
| 15 | O-2268           | pAG413GPD-NLS-Sis1-EGFP      |
| 16 | O-2283           | pAG413GPD-NES-Sis1-EGFP      |
| 17 | O-2269           | pAG415GPD-NLS-Sis1-mCherry   |
| 18 | O-2272           | pAG415GPD-NLS-Sis1-mCherry   |
| 19 | O-2135           | pAG416GAL-Sis1-HA             |
| 20 | O-2298           | pAG416GAL-NLS-Sis1-HA        |
| 21 | O-2299           | pAG416GAL-NE-Sis1-HA         |
|   |   |
|---|---|
| 22 | O-2223 | pAG416GPD-Nrp1PrD-mCherry |
| 23 | O-2094 | pAG416GAL-Nrp1PrD-EGFP |
| 24 | O-576  | pAG425GAL-Nrp1PrD-EYFP |
| 25 | O-2225 | pAG416GPD-Nrp1PrD-FLAG |
| 26 | O-1354 | pAG415GPD-Rnq1PrD-mCherry |
| 27 | O-2206 | pAG416GPD-ymOrange-FLAG |
| 28 | O-2098 | pAG415GPD-ymOrange-HA |
| 29 | O-2102 | pAG415GPD-yEGFP |
| 30 | O-2104 | pAG416GPD-yEGFP |
| 31 | O-2151 | pAG415GAL-yEGFP |
| 32 | O-2152 | pAG416GAL-yEGFP |
| 33 | O-2148 | pAG415GPD-Btn2 |
| 34 | O-1979 | pAG416GPD-Btn2 |
| 35 | O-1977 | pAG416GAL-Btn2 |
| 36 | O-1981 | pAG426GAL-Btn2 |
| 37 | O-2096 | pAG415GPD-Btn2-HA |
| 38 | O-1947 | pAG304GAL-Btn2-HA |
| 39 | O-2317 | pAG415GPD-Btn2-EGFP |
| 40 | O-2089 | pAG416GPD-Btn2-EGFP |
| 41 | O-2178 | pAG416GPD-Btn2ΔNLS-EGFP |
| 42 | O-2180 | pAG416GAL-Btn2ΔNLS |
| 43 | O-2181 | pAG426GAL-Btn2ΔNLS |
| 44 | O-2203 | pAG416GPD-Btn2-FLAG |
| 45 | O-2149 | pAG415GPD-Cur1 |
| 46 | O-1980 | pAG416GPD-Cur1 |
| 47 | O-1978 | pAG416GAL-Cur1 |
| 48 | O-1982 | pAG426GAL-Cur1 |
| 49 | O-2097 | pAG415GPD-Cur1-HA |
| 50 | O-1948 | pAG304GAL-Cur1-HA |
| 51 | O-2319 | pAG415GPD-Cur1-EGFP |
| 52 | O-2090 | pAG416GPD-Cur1-EGFP |
| 53 | O-2091 | pAG416GPD-Cur1ΔNLS-EGFP |
| 54 | O-2066 | pAG416GAL-Cur1ANLS |
| 55 | O-2070 | pAG426GAL-Cur1ANLS |
| 56 | O-2201 | pAG416GPD-Cur1-FLAG |
| 57 | O-1360 | pAG415GDP-Hsp104-mCherry |
| 58 | O-1465 | pAG415GPD-Sgt2-mCherry |
| 59 | O-2252 | pAG415GDP-Hsp42-mCherry |
| 60 | O-1361 | pAG415GDP-Hsp26-mCherry |
| 61 | O-1499 | pAG416GAL-Aha1 |
| 62 | O-1193 | pAG416GAL-Cns1 |
| 63 | O-1191 | pAG416GAL-Cpr7 |
| 64 | O-1192 | pAG416GAL-Stl1 |
| 65 | O-1334 | pAG416GAL-Sgt1 |
| 66 | O-1335 | pAG416GAL-Sgt2 |
| 67 | O-1184 | pAG416GAL-Fes1 |
| 68 | O-1183 | pAG416GAL-Sse1 |
| 69 | O-1185 | pAG416GAL-Hsp26 |
| 70 | O-1557 | pAG416GAL-Hsp42 |
| 71 | O-1186 | pAG416GAL-Hsp104 |
| 72 | O-1972 | pAG416GAL-Ydj1 |
| 73 | O-1175 | pAG416GAL-Sis1 |
DNA synthesis

Variant versions of Sis1 were synthesized and assembled by Geneart (Invitrogen) and then cloned into the pDONR221 plasmid. The coding sequences were codon-optimized for expression in yeast and contained flanking sequences that allowed for Gateway® recombination and dual expression in yeast and bacteria (the recombinogenic attB sites is highlighted in blue, the Shine-Dalgarno ribosome binding site in red and the yeast Kozak consensus sequence in green):

**DNA sequence of NLS-SIS1**

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ACAAAGTTTGTACAAAAAAACAGGCTTCGTTTGAATTGGTCAAAGAAA
CTAAGTTGTACGACTTGTTGGGTGTTTCTCCATCTGCTAATGAACAAGAATT
GAAGAAGGGTTACAGAAAGGCTGCTTTGAAATACCATCCAGATAAGCCAAC
TGGTGATACCGAAAA
```
Translation of NLS-Sis1 (SV40 NLS is shown in red):

M\text{AEILIPEPPKKRKVEL}VKETKLYDLLGVSPSANEQELKKGKYRKAALKYHPDKP
TGDETFKKEISEAFEILNDPQKREIYDQYGLEAARSGGPSFGPGPGPAGGAG
GFPGGAGGFSGGHAFSNEDAFNIFSQFGGGSSPGGADDGSFGFSSSYPGGG
AGMGGMPGGMGMHMGGMGMPGFRSASSSPTYPEEETVQVNLPVSLEDL
FVGKKSFKIKRGKGASEKTQIDIQLKPGKWAGTKITYKNGQGDYNQPRR
KTLQFVIQEKSHPNFKRDGDDL1YTPLSFKESLLGFSKTIQDGRIDRTLPSRVPQ
VQPSQSTYPQGMPTPKNPSQRGLNVKDYPIGLNDAQKRAIDENF

Antibodies

Antibodies were purchased from different vendors. Table S2 contains a list of the antibodies that were used.

Table S2: Antibodies used in this study.

| # | Antibody   | Company name  | Catalog number |
|---|------------|---------------|----------------|
| 1 | Anti-GST   | Thermo scientific | CAB4169       |
| 2 | Anti-ubiquitin | Dako       | Z0458          |
| 3 | Anti-PGK   | Invitrogen    | 459250         |
| 4 | Anti-GFP   | Roche        | 11814460001    |
| 5 | Anti-HA    | Covance      | MMS-10P        |
| 6 | Anti-FLAG (M2) | Sigma-Aldrich | F1804-200UG    |
### Yeast strains

Table S3: Yeast strains used in this study.

| #  | Number | Background | Genotype |
|----|--------|------------|----------|
| 1  | YAL-456| YRS100     | sup35::HygB; pAG415ADH1-NRP1PrD-SUP35C; [NRP1-C+] |
| 2  | YAL-887| YRS100     | sup35::HygB; pAG415ADH1-NRP1PrD-SUP35C; [nrp1-c+]; hsp104::SpHis5 |
| 3  | YAL-414| YRS100     | sup35::HygB; pAG415ADH1-NRP1PrD-SUP35C; [nrp1-c-] |
| 4  | YAL-1504| YRS100    | sup35::HygB; pAG415ADH1-NRP1PrD-SUP35C; [NRP1-C+]; cur1::SpHis5 |
| 5  | YAL-1487| YRS100    | sup35::HygB; pAG415ADH1-NRP1PrD-SUP35C; [NRP1-C+]; btn2::SpHis5 |
| 6  | YAL-1487| YRS100    | sup35::HygB; pAG415ADH1-NRP1PrD-SUP35C; [NRP1-C+]; Δyrp158w; btn2::SpHis5 |
| 7  | YAL-2171| YRS100    | sup35::HygB; pAG415ADH1-NRP1PrD-SUP35C; sis1::KanMX, pAG413MET3-Sis1; [NRP1-C+] |
| 8  | YAL-948| BY4741     | SIS1-GFP::His3MX; [rq-] |
| 9  | YAL-215| BY4741     | SIS1-GFP::His3MX; [RNQ+] |
| 10 | YAL-1692| BY4741    | BTN2-YFP::His3MX |
| 11 | YAL-2150| CIM3-1    | CUR1-yEGFP::KanMX |
| 12 | YAL-1317| BY4741    | ydj1::KanMX4; [rq-] |
| 13 | YAL-1608| BY4741    | ydj1::KanMX4; btn2::SpHis5; [rq-] |
| 14 | YAL-1609| BY4741    | ydj1::KanMX4; cur1::SpHis5; [rq-] |
| 15 | YAL-1610| BY4741    | ydj1::His3MX; Δbtn2; cur1::KanMX; [rq-] |
| 16 | YAL-1303| BY4741    | btn2::KanMX4; [rq-] |
| 17 | YAL-1309| BY4741    | cur1::KanMX4; [rq-] |
| 18 | YAL-1357| BY4741    | btn2::KanMX4; cur1::HygB; [rq-] |
| 19 | YAL-1361| BY4741    | cur1::KanMX4; btn2::HygB; [rq-] |
| 20 | YAL-1375| BY4741    | SIS1-GFP::His3MX; btn2::KanMX; cur1::HygB; [rq-] |
| 21 | YAL-1383| BY4741    | SIS1-GFP::His3MX; btn2::HygB; [rq-] |
| 22 | YAL-1381| BY4741    | SIS1-GFP::His3MX; cur1::HygB; [rq-] |
| 23 | YAL-1521| BY4741    | pre9::KanMX4; [RNQ+] |
| 24 | YAL-1781| BY4741    | sis1::SpHis5; 415ADH1-Sis1-EGFP |
| 25 | YAL-1789| BY4741    | sis1::SpHis5; 415GPD-Sis1ΔC-EGFP |
| 26 | YAL-1639| PRE1-1     | btn2::KanMX |
| 27 | YAL-1612| PRE1-1     | btn2::KanMX; cur1::SpHis5 |
| 28 | YAL-1615| PRE1-1     | cur1::KanMX |
| 29 | YAL-1617| PRE1-1     | btn2::KanMX; cur1::SpHis5 |
| 30 | YAL-1885| BY4741    | SIS1-GFP::His3MX; HSP104-tdimer2::KanMX |
| 31 | YAL-1883| BY4741    | SIS1-GFP::His3MX; HSP42-tdimer2::KanMX |
| 32 | YAL-1889| BY4741    | SIS1-GFP::His3MX; PRE6-tdimer2::KanMX |
| 33 | YAL-1887| BY4741    | SIS1-GFP::His3MX; RPN1-tdimer2::KanMX |
| 34 | YAL-1291| BY4741    | SSA1-GFP::His3MX; [rq-] |
| 35 | YAL-1295| BY4741    | HSP42-GFP::His3MX; [rq-] |
| 36 | YAL-2072| W303      | PRE6-tdimer2::KanMX |
| 37 | YAL-2074| W303      | HSP104-tdimer2::KanMX |
| 38 | YAL-2071| W303      | HSP42-tdimer2::KanMX |
SUPPORTING FIGURES

Figure S1. Stress-inducible Btn2 and Cur1 interfere with prion inheritance. (A) [NRP1C+] yeast were treated with 5 mM guanidine hydrochloride or transformed with knock out cassettes for HSP104 or RNQ1. The resulting strains were spotted onto plates containing rich medium. Untreated [NRP1C+] or [nrp1c-] cells are shown for comparison. (B) Galactose-regulatable expression plasmids coding for the indicated yeast proteins were introduced into [NRP1C+] yeast. The transformants were transferred onto galactose-containing plates and incubated for three days at 30°C. (C) Chromosomal BTN2 was tagged with GFP in the BY4741 strain background. The strain was grown over night at 25°C and then incubated for an additional hour at 25°C or 39°C. Cell lysates were prepared and analyzed by immunoblotting with an anti-GFP antibody. Pgp1 was detected with a specific antibody and served as a loading control. (D) GFP-tagged Btn2, Cur1 and Sis1 were expressed in a BY4741 wildtype strain and a strain that carried a
deletion of \textit{PRE9}. Proteins were detected by immunoblotting with a GFP-specific antibody. Pgk1 served as a loading control. (E) Same as (C) except that \textit{CUR1} was modified with GFP in a strain carrying a temperature-sensitive mutation in the proteasome subunit \textit{CIM3}. (F) BY4741 yeast expressing a GFP-tagged chromosomal copy of Hsp104 were grown at 37°C for 1 h and processed for immunoblotting with specific antibodies for GFP, Ssa1/2, Sis1 and Pgk1. The steady state levels of the indicated proteins are compared in wildtype, \Delta btn2, \Delta cur1 and \Delta btn2 \Delta cur1 cells.

**Figure S2.** Btn2 and Cur1 functionally and physically interact with Sis1 to modify prion inheritance. (A) Galactose-regulatable expression plasmids coding for the indicated yeast proteins were introduced into [\textit{NRP1C+}] yeast. The transformants were transferred onto galactose-containing plates and incubated for three days at 37°C. (B) [\textit{NRP1C+}] strains containing galactose-regulatable expression cassettes for Btn2 or Cur1 or EGFP (control) were transformed with a low copy plasmid for Sis1 expression. The transformants were streaked onto plates containing glucose (YPD) or galactose (YPGal) and incubated at 30°C for three days. (C) Endogenous SIS1 was replaced with a GAL-\textit{SIS1} construct in BY4741 yeast cells containing an integrated expression cassette for Nrp1PrD-EGFP. The cells were grown in the presence of galactose (high Sis1 concentration) or glucose (low Sis concentration) and the fraction of cells with visible aggregates was determined. (D) Gel filtration of yeast cell lysates from [\textit{NRP1C+}] cells that expressed Sis1 from a low copy expression plasmid. Protein fractions were
applied onto a Protran nitrocellulose filter by using a dot blot apparatus. Nrp1PrD-Sup35C was detected with a Sup35-specific antibody. Molecular weight markers were: thyroglobulin (660kDa), ferritin (440kDa), catalase (230kDa), aldolase (160kDa), bovine serum albumin (67kDa) and ovalbumin (43kDa). (E) Fluorescence microscopy of BY4741 yeast cells expressing Nrp1PrD-mCherry from a low copy plasmid and Sis1-GFP from the chromosomal locus at 25°C. (F) FLAG-tagged Orange (control) and Nrp1PrD were expressed in a BY4741 strain carrying a GFP-tagged chromosomal copy of SIS1. Proteins were immunoprecipitated using an anti-FLAG antibody. The asterisk marks a degradation product of Orange. Due to the relatively lower expression level, Nrp1PrD was only detected in the total after longer exposure times (data not shown). (G) [NRP1C+] cells containing deletions ($\Delta btn2$, $\Delta cur1$ and $\Delta btn2 \Delta cur1$) or expressing additional Sis1 from a low copy plasmid were processed for SDD-AGE. Immunoblotting was performed with a Sup35-specific antibody.

**Figure S3.** Btn2 and Cur1 promote the sorting of Sis1 to the nucleus and to stress-inducible cytosolic compartments. (A) Low copy expression plasmids for Btn2 and Cur1 were introduced into a BY4741 strain that expressed GFP-tagged Sis1 from the endogenous locus. The strains were grown at 25°C and were subjected to fluorescence microscopy. The average nuclear and cytosolic GFP pixel intensity was obtained from digital images of 50 cells per strain. The y-axis gives the nuclear:cytosolic ratio of the GFP pixel intensity. Error bars denote the standard error of the mean ($^* \ p = 1.3 \times 10^{-13}$, $^{**} \ p = 3.7 \times 10^{-20}$). (B) Low copy
expression plasmids for Btn2 and Cur1 were introduced into a BY4741 strain expressing GFP-tagged Sis1. The transformants were grown at 25°C and subjected to fluorescence microscopy. The fraction of foci-containing cells was determined. At least 195 cells per strain were examined. (C) Top: schematic representation of the domain organization of Sis1. Bottom: Btn2 or Cur1 were co-expressed with wildtype or mutant versions of Sis1-GFP. Images on the left show the localization of Sis1-GFP, while images on the right show an overlay with a mCherry-tagged nuclear marker. (D) BY4741 yeast cells were transformed with low copy expression plasmids for the indicated proteins. FLAG-tagged proteins were immunoprecipitated from cell lysate with a FLAG-specific antibody. Proteins were detected by immunoblotting with antibodies against GFP and the FLAG epitope. (E) Same as (C), except that the proteins were immunoprecipitated using an antibody specific for GFP.

**Figure S4.** Nuclear targeting of Sis1 is dependent on nuclear localization sequences in Btn2 and Cur1 and requires the α-importin Srp1. (A) Quantification of the relative nuclear:cytosolic GFP pixel intensity of the strains that expressed GFP-tagged Btn2, Btn2ΔNLS, Cur1 or Cur1ΔNLS (* p = 2.1 x 10^{-14}; ** p = 4.0 x 10^{-29}). We refer the reader to the materials and methods section for details on image acquisition and quantification. The analyzed images were acquired at 25°C to minimize the number of fluorescent foci in the cytosol. (B) BY4741 yeast cells were transformed with low copy expression plasmids for the indicated proteins. FLAG-tagged proteins were immunoprecipitated from cell lysate with a
FLAG-specific antibody. Proteins were detected by immunoblotting with antibodies against GFP and the FLAG epitope. The asterisk denotes the heavy chain of the antibody that was used for immunoprecipitation. (C) Low copy expression plasmids coding for GFP-tagged Btn2, Btn2ΔNLS, Cur1 or Cur1ΔNLS were introduced into BY4741 yeast. The transformants were processed for immunoblotting with a GFP-specific antibody. Pgk1 served as a loading control. (D) Wildtype yeast ('WT') or yeast carrying a temperature-sensitive mutation in SRP1 ('srp1-31') were co-transformed with expression plasmids for Sis1-GFP and Orange (control), Btn2 or Cur1. The cells were subjected to fluorescence microscopy after a shift to the non-permissive temperature for 1 h. (E) Yeast cells carrying a GFP-tagged chromosomal copy of SRP1 were transformed with low copy expression plasmids for HA-tagged Sis1 and FLAG-tagged Orange (control), BTN2 or CUR1. FLAG-tagged proteins were immunoprecipitated with a specific antibody. Because of a strong signal, the anti-FLAG immunoblot on the top right received only 1/10 of the control (Orange-FLAG) sample. The asterisk denotes a band that was produced by the heavy chain of the antibody. (F) Protein binding assay with bacterially purified GST-Btn2, GST-Btn2ΔNLS, GST-Cur1, GST-Cur1ΔNLS and His6-Srp1. Proteins were detected by immunoblotting with an anti-GST or anti-His antibody. The pull down efficiency was ~20% for the GST-tagged proteins. 2.5% of the input is shown for comparison.

**Figure S5.** Complex formation between Sis1 and Btn2 or Cur1 is required for targeting to the nucleus. (A) Quantification of the relative nuclear:cytosolic GFP
pixel intensity of the strains shown in Figure 5E. The average GFP pixel intensity
was obtained from 50 cells. Error bars represent the standard error of the mean
(* p = 4.0 \times 10^{-14}; ** p = 2.9 \times 10^{-18}; *** p = 3.4 \times 10^{-22}; **** p = 5.6 \times 10^{-16}).

**Figure S6.** Localization of Btn2 to a peripheral compartment is dependent on
Hsp42. (A) Fluorescence microscopy of BY4741 yeast expressing Btn2-GFP and
mCherry (control), Hsp104-mCherry, Hsp42-mCherry or Hsp26-mCherry from a
plasmid at 25°C. (B) Fluorescence microscopy of BY4741 yeast cells expressing
Hsp42-mCherry and Btn2ΔNLS-GFP or Cur1ΔNLS-GFP at 25°C. The NLS-
deleted versions were used to ensure that only the peripheral compartment was
formed.

**Figure S7.** Btn2 promotes the sorting of misfolded proteins to cytosolic protein
deposition sites. (A) Left: A low copy expression plasmid coding for GFP-VHL
was introduced into BY4741 wildtype and Δhsp42 yeast. The cells were grown at
25°C, shifted to 37°C in the presence of MG132 for 1 hour and subjected to
fluorescence microscopy. The fraction of foci-containing cells was determined. At
least 179 cells were analyzed per strain. Right: cells expressed GFP-Ubc9ts
instead of GFP-VHL. At least 230 cells were analyzed per strain. (B) Low copy
expression plasmids for GFP-VHL or GFP-Ubc9ts were introduced into a
wildtype strain or a strain lacking functional Hsp42. Cells were grown at 25°C,
shifted to 37°C for 1 h in the presence of MG132 and observed by fluorescence
microscopy. (C) BY4741 yeast cells carrying a GFP-tagged chromosomal copy of
HSP42 were transformed with an expression plasmid for Btn2. Cell lysates were prepared and analyzed by immunoblotting with a GFP-specific antibody. The GFP signal was detected using the Chemismart 5100 chemiluminescence imaging system. GFP bands were quantified using Fiji and normalized against Pgk1. The graph shows the average relative intensity of three independent experiments. (D) BY4741 yeast expressing GFP-VHL and FLAG-tagged Orange (control), Sis1 or Sis1ΔC were lysed and GFP-VHL was immunoprecipitated from the cell lysates with a GFP-specific antibody. The asterisks denote degradation products.

**Figure S8.** Btn2 and Cur1 influence prion propagation indirectly through changes in the availability of Sis1. (A) Wildtype [NRP1C+] cells or cells in which chromosomal Sis1 was replaced with Sis1ΔDD were incubated on YPD plates at 30°C for three days. (B) Low copy expression plasmids for GFP-tagged SIS1 or NLS-SIS1 were introduced into a BY4741 strain that expressed a nuclear marker. Cells were observed by fluorescence microscopy at 25°C. (C) Left: low copy expression plasmids for expression of Sis1 or NLS-Sis1 were introduced into a BY4741 strain that expressed GFP-tagged Sis1 from the endogenous locus. Nab2NLS-2mCherry was used as a marker for the nucleus. The cells were observed by fluorescence microscopy at 25°C. Right: Quantification of the relative nuclear:cytosolic GFP pixel intensity. The average GFP pixel intensity was obtained from 50 cells. Error bars represent the standard error of the mean (* p = 4.5 x 10^{-24}). (D) [NRP1C+] cells were transformed with galactose-
regulatable expression plasmids for Btn2, Btn2ΔNLS, Cur1 or Cur1ΔNLS. The transformants were streaked onto galactose plates, incubated for 3 days and transferred onto YPD plates for color development.

**Figure S9.** Cur1 regulates the partitioning of substrate proteins between the juxtanuclear and peripheral compartments. (A) BY4741 yeast cells were transformed with low-copy or high-copy galactose-regulatable expression plasmids for BTN2 and CUR1. The transformants were grown overnight in glucose-containing media. Fivefold serial dilutions were prepared and spotted onto either glucose- (repressing) or galactose-containing (inducing) plates. The plates were incubated at 30°C for three days. (B) Ydj1-deficient yeast cells were transformed with a plasmid for constitutive Sis1 expression and galactose-regulatable expression plasmids for Btn2 and Cur1. The cells were spotted onto either glucose- or galactose-containing plates. The plates were incubated at 30°C. (C) Cells were treated as in (B) using plasmids for the indicated proteins. (D) Wildtype yeast and yeast with a mutation in PRE1 were transformed with low copy galactose-regulatable expression plasmids for Sis1 or NLS-Sis1. Fivefold serial dilutions of over night cultures were spotted onto either glucose- or galactose-containing plates. The plates were incubated at 30°C for three days. (E) BY4741 yeast cells carrying a GFP-tagged chromosomal copy of HSP42 were transformed with an expression plasmid for Cur1. Cell lysates were prepared and analyzed by immunoblotting with a GFP-specific antibody. The GFP signal was detected using the Chemismart 5100 chemiluminescence
imaging system. GFP bands were quantified using Fiji and normalized against Pgk1. The graph shows the average relative intensity of three independent experiments.

**Movie S1.** Wildtype, Δbtn2, Δcur1 or Δbtn2 Δcur1 BY4741 yeast cells expressing Sis1-GFP from the chromosomal locus and a mCherry-tagged nuclear marker were incubated at the indicated temperatures in the presence of the proteasome inhibitor MG-132. MG132 was added at a final concentration of 20 µM when indicated in the movie. In the recovery phase MG132 was washed out. After the addition and removal of MG132 a different field of view is shown. Please note that the cells in the different movies were exposed to identical conditions, as the images were acquired in the same experiment.

**Movie S2.** Wildtype, Δbtn2, Δcur1 or Δbtn2 Δcur1 BY4741 yeast cells expressing Sis1-GFP from the chromosomal locus and mCherry-VHL from a low copy plasmid were exposed to the indicated temperatures. MG-132 was added at a final concentration of 20 µM when indicated in the movie. Please note that the cells in the different movies were exposed to identical conditions, as the images were acquired in the same experiment.

**Movie S3.** BY4741 yeast cells expressing Sis1-GFP from the chromosomal locus and a mCherry-tagged nuclear marker were incubated at 38°C for 3 hours in the presence of the proteasome inhibitor MG-132. After the heat shock the
temperature returned to 25°C and MG132 was washed out. The movie starts at the beginning of the recovery phase.

**SUPPORTING REFERENCES**

1. Brameier M, Krings A, MacCallum RM (2007) NucPred--predicting nuclear localization of proteins. Bioinformatics 23: 1159-1160.
