Supporting Information

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

Liliana Malinovska, Sonja Kroschwald, Matthias Munder, Doris Richter, Simon Alberti

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):

MFSIFNSPCVFEQLPSFSQPLHSRYFDCSSPVSYYPECKRRKAIKANLRAPKKS
DANCSEPLRYALAETPNGYTLSLSKRIPYELFSKYVNEKLGELKENHYRPTYHV
VQDFFGNQYYVEDEADEDALLRSALKDLDFRAIGKKIAKDLFQDYEIELNHRGD
ELSILSKKDKIFKEFSLDQVFEDVFVIGCGVENIDDGSREKYALLKIGLVKHEEEIS
EGGINEPKMPIIESKIDESHDDVNMSESLKEEEAEKAKEPLTKEDQIKKWIEEER
LMQEESRKSEQEKAAKEDEERQKKEKEARLKARKESLINKQKTKRSQQKKLQN
SKSLPISEIEASNKNNNSNSGSAESDNESINSDSDTTLDFSVSGNTLKKHASPLL
EDVEDEEVDRYNESLSRSPKGNSIIEEI

Results for Cur1 (NucPred score 0.85):

MAAACICQPNLLEINVSDGPLDMIRKKRKIQQPQLRPPLRENKCQPHFSVRKVN
QSYIISLHKEITCQLIAEIVKQKLSRIWEKVYIPSYELISDKDGNQIYVEQSVDENR
LTSEIMEKLDPNNIDIEAIEILFDDYHLELSRLTNGIIISSANDHFYREFSFNNIIDDN

FKICGTSMSADSFDKIYGVMWIEVPFNGNGLQNDSAVNRVSTSHNQIEELNDIE
QEIRAFNISRSNQESIIKKEVSRRLNGR

Results for Sis1 (NucPred score 0.64):

MVKETKLYDLLGVSPSANEQELKKGYRKAALKYHPDKPTGDTEKFKEISEAFEIL
NDPQKREIYDQYGLEAARSGGPSFGPGGPGGAGGAGGFPGGAGGFSGGHAF
SNEDAFNIFSQFFGGSSPFGGADDSGFSFSSYPSGGGAGMGGMPGGMGGM
HGGMGGMPGGFRSASSSPTYPEEETVQVNLPVSLEDLFVGKKKSFKIGRKGP
HGASEKTQIDIQLKPGWKAGTKITYKNQGDYNPQTGRRKTLQFVIQEKSHPNFK
RDGDDLIYTLPLSFKESLLGFSKTIQTIDGRTLPLSRVQPVQPSQTSTYPGQGMP
TPKNPSQRGNLIVKYKVDYPISLNDAQKRAIDENF

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-mCherry
9	O-2196	pAG415ADH1-Sis1ΔC-EGFP
10	O-2213	pAG415GPD-Sis1ΔC-EGFP
11	O-2229	pAG425GPD-Sis1ΔC-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-NES-Sis1-mCherry
19	O-2135	pAG416GAL-Sis1-HA
20	O-2298	pAG416GAL-NLS-Sis1-HA
21	O-2299	pAG416GAL-NES-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-Cur1ΔNLS
55	O-2070	pAG426GAL-Cur1ΔNLS
56	O-2201	pAG416GPD-Cur1-FLAG
57	O-1360	pAG415GPD-Hsp104-mCherry
58	O-1465	pAG415GPD-Sgt2-mCherry
59	O-2252	pAG415GPD-Hsp42-mCherry
60	O-1361	pAG415GPD-Hsp26-mCherry
61	O-1499	pAG416GAL-Aha1
62	O-1193	pAG416GAL-Cns1
63	O-1191	pAG416GAL-Cpr7
64	O-1192	pAG416GAL-Sti1
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

74	O-1188	pAG416GAL-Ssb1
75	O-1182	pAG416GAL-Ssb2
76	O-1190	pAG416GAL-Ssa1
77	O-1187	pAG416GAL-Ssa2
78	O-1503	pAG416GAL-Ssa3
79	O-1498	pAG416GAL-Ssa4
80	O-1151	pAG416GAL-Hsc82
81	O-1150	pAG416GAL-Hsp82
82	O-2335	pAG415GPD-Hsp42
83	L-200	Nab2NLS-2mCherry pYX242 (M. Route)
84	L-9	pESC-URA-mCherry-VHL (J. Frydman)
85	L-157	pESC-LEU-GFP-VHL (J. Frydman)
86	L-155	pESC-URA-GFP-Ubc9ts (J. Frydman)
87	O-2304	pDEST15-Btn2-dNLS
88	O-2305	pDEST17-Srp1
89	O-2122	pDEST15-Cur1
90	O-2121	pDEST15-Btn2
91	O-2119	pDEST15-EGFP
92	O-2056	pRH1-Sis1
93	O-2172	pDEST15-Cur1ΔNLS
94	O-2120	pDEST15-Sis1
95	O- 2532	pAG415ADH1-Sis1(K199A)-EGFP
96	O-2533	pAG415ADH1-Sis1ΔDD-EGFP
97	O-2534	pAG415ADH1-Sis1(H34Q)-EGFP

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

ACAAGTTTGTACAAAAAAGCAGGCTTCGAAGGAGATAACAAAATGGCTGAAT
TGATTCCAGAGCCACCAAAAAAGAAGAGAAAGGTTGAATTGGTCAAAGAAA
CTAAGTTGTACGACTTGTTGGGTGTTTCTCCATCTGCTAATGAACAAGAATT
GAAGAAGGGTTACAGAAAGGCTGCTTTGAAATACCATCCAGATAAGCCAAC
TGGTGATACCGAAAAGTTCAAAGAAATTTCCGAAGCCTTCGAGATCTTGAAT

GATCCACAAAAGAGGGAAATCTACGACCAATATGGTTTGGAAGCTGCTAGA
TCTGGTGGTCCATCTTTTGGTCCAGGTGGTCCTGGTGGTGCAGGCGGTGCT
GGTGGTTTTCCAGGTGGTGCTGGCGGTTTCTCTGGTGGTCATGCTTTTTCTA
ATGAAGATGCCTTCAACATCTTCTCCCAATTTTTTGGTGGTTCTTCTCCATTT
GGTGGTGCTGATGATTCTGGTTTTTCTTTTTCTTCATACCCATCTGGTGGTG
GTGCTGGTATGGGTGGTATGCCAGGTGGTATGGGAGGAATGCATGGTGGA
ATGGGTGGCATGCCTGGCGGTTTTAGATCTGCTTCTTCTTCACCAACTTACC
CAGAAGAAGAAACCGTTCAAGTTAATTTGCCAGTCTCCTTGGAAGATTTGTT
CGTTGGTAAAAAGAAGTCCTTCAAGATCGGTAGAAAAGGTCCACATGGTGC
TTCAGAAAAGACCCAAATTGACATTCAATTGAAGCCAGGTTGGAAAGCTGGT
ACTAAGATTACCTACAAGAACCAGGGTGATTACAATCCACAAACTGGTAGAA
GAAAGACCTTGCAATTCGTCATTCAAGAAAAGTCCCACCCAAATTTCAAGAG
GGATGGTGATGATTTGATCTACACTTTGCCATTGTCCTTCAAAGAATCCTTGT
TGGGTTTCTCCAAGACCATTCAAACCATTGATGGTAGAACCTTGCCATTGTC
TAGAGTTCAACCTGTTCAACCATCTCAAACTTCTACTTATCCAGGTCAAGGT
ATGCCAACTCCAAAAAATCCATCTCAAAGGGGTAACTTGATCGTTAAGTACA
AGGTTGATTACCCAATCTCCTTGAACGATGCTCAAAAAAGAGCCATTGACGA
GAACTTTAACCCAGCTTTCTTGTACAAAGTGGT

Translation of NLS-Sis1 (SV40 NLS is shown in red):

MAELIPEPPKKKRKVELVKETKLYDLLGVSPSANEQELKKGYRKAALKYHPDKP
TGDTEKFKEISEAFEILNDPQKREIYDQYGLEAARSGGPSFGPGGPGGAGGAG
GFPGGAGGFSGGHAFSNEDAFNIFSQFFGGSSPFGGADDSGFSFSSYPSGGG
AGMGGMPGGMGGMHGGMGGMPGGFRSASSSPTYPEEETVQVNLPVSLEDL
FVGKKKSFKIGRKGPHGASEKTQIDIQLKPGWKAGTKITYKNQGDYNPQTGRR
KTLQFVIQEKSHPNFKRDGDDLIYTLPLSFKESLLGFSKTIQTIDGRTLPLSRVQP
VQPSQTSTYPGQGMPTPKNPSQRGNLIVKYKVDYPISLNDAQKRAIDENF

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

7	Anti-His6Tag	Dianova	Dia 900
8	Anti-Ssa1/2	Santa Cruz	Sc-23752

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-1485	YRS100	sup35::HygB; pAG415ADH1-NRP1PrD-SUP35C; [NRP1-C+]; Δypr158w; btn2::SpHis5
7	YAL-2171	YRS100	sup35::HygB; pAG415ADH1-NRP1PrD-SUP35C; sis1::KanMX, pAG413MET3-Sis1; [NRP1-C+]
8	YAL-948	BY4741	SIS1-GFP::His3MX; [rnq-]
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; [rnq-]
13	YAL-1608	BY4741	ydj1::KanMX4; btn2::SpHis5; [rnq-]
14	YAL-1609	BY4741	ydj1::KanMX4; cur1::SpHis5; [rnq-]
15	YAL-1610	BY4741	ydj1::His3MX; Δbtn2; cur1::KanMX; [rnq-]
16	YAL-1303	BY4741	btn2::KanMX4; [rnq-]
17	YAL-1309	BY4741	cur1::KanMX4; [rnq-]
18	YAL-1357	BY4741	btn2::KanMX4; cur1::HygB; [rnq-]
19	YAL-1361	BY4741	cur1::KanMX4; btn2::HygB; [rnq-]
20	YAL-1375	BY4741	SIS1-GFP::His3MX; btn2::KanMX; cur1::HygB; [rnq-]
21	YAL-1383	BY4741	SIS1-GFP::His3MX; btn2::HygB; [rnq-]
22	YAL-1381	BY4741	SIS1-GFP::His3MX; cur1::HygB; [rnq-]
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	
27	YAL-1612	PRE1-1	btn2::KanMX
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; [rnq-]
35	YAL-1295	BY4741	HSP42-GFP::His3MX; [rnq-]
36	YAL-2072	W303	PRE6-tdimer2::KanMX
37	YAL-2074	W303	HSP104-tdimer2::KanMX
38	YAL-2071	W303	HSP42-tdimer2::KanMX

39	YAL-2076	W303	RPN1-tdimer2::KanMX
40	YAL-1777	BY4741	sis1::SpHis5; 415GPD-Sis1-mCherry
41	YAL-2198	BY4741	sis1::SpHis5; 425GPD-Sis1ΔC-mCherry
42	YAL-1285	BY4741	HSP104-GFP::His3MX; [rnq-]
43	YAL-1308	BY4741	hsp42::KanMX4; [rnq-]
44	YAL-2137	BY4741	Δbtn2; hsp42::KanMX4; [rnq-]
45	YAL-2139	BY4741	Δcur1; hsp42::KanMX4; [rnq-]
46	YAL-2201	BY4741	Δbtn2; Δcur1; hsp42::KanMX4; [rnq-]
47	YAL-1345	YRS100	sup35::HygB; pAG415ADH1-NRP1PrD-SUP35C; pAG304GAL-EGFP-HA; [NRP1-C+]
48	YAL-1349	YRS100	sup35::HygB; pAG415ADH1-NRP1PrD-SUP35C; pAG304GAL-BTN2-HA; [NRP1-C+]
49	YAL-1353	YRS100	sup35::HygB; pAG415ADH1-NRP1PrD-SUP35C; pAG304GAL-CUR1-HA; [NRP1-C+]
50	YAL-2204	W303	HSP42-tdimer2::KanMX; pAG304GPD-SIS1
51	YAL-2094	BY4741	srp1::CUP1-yEGFP-SRP1
52	YAL-1964	SRP1-31	

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. Pgk1 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 *PRE9*. Proteins were detected by immunoblotting with a GFP-specific antibody. Pgk1 served as a loading control. (E) Same as (C) except that *CUR1* was modified with GFP in a strain carrying a temperature-sensitive mutation in the proteasome subunit *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, Δ*btn2*, Δ*cur1* and Δ*btn2* Δ*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 [*NRP1C+*] yeast. The transformants were transferred onto galactose-containing plates and incubated for three days at 37°C. (B) [*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-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 [*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 (Δ*btn2*, Δ*cur1* and Δ*btn2* Δ*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 (* $p = 2.1 \times 10^{-14}$; ** $p = 4.0 \times 10^{-29}$). 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 Δ*hsp42* 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 (* $p = 4.5 \times 10^{-24}$). (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 hock the

temperature returned to 25°C and MG132 was washed out. The movie starts at the beginning of the recovery phase.

SUPPORTING REFRENCES

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

Figure S1

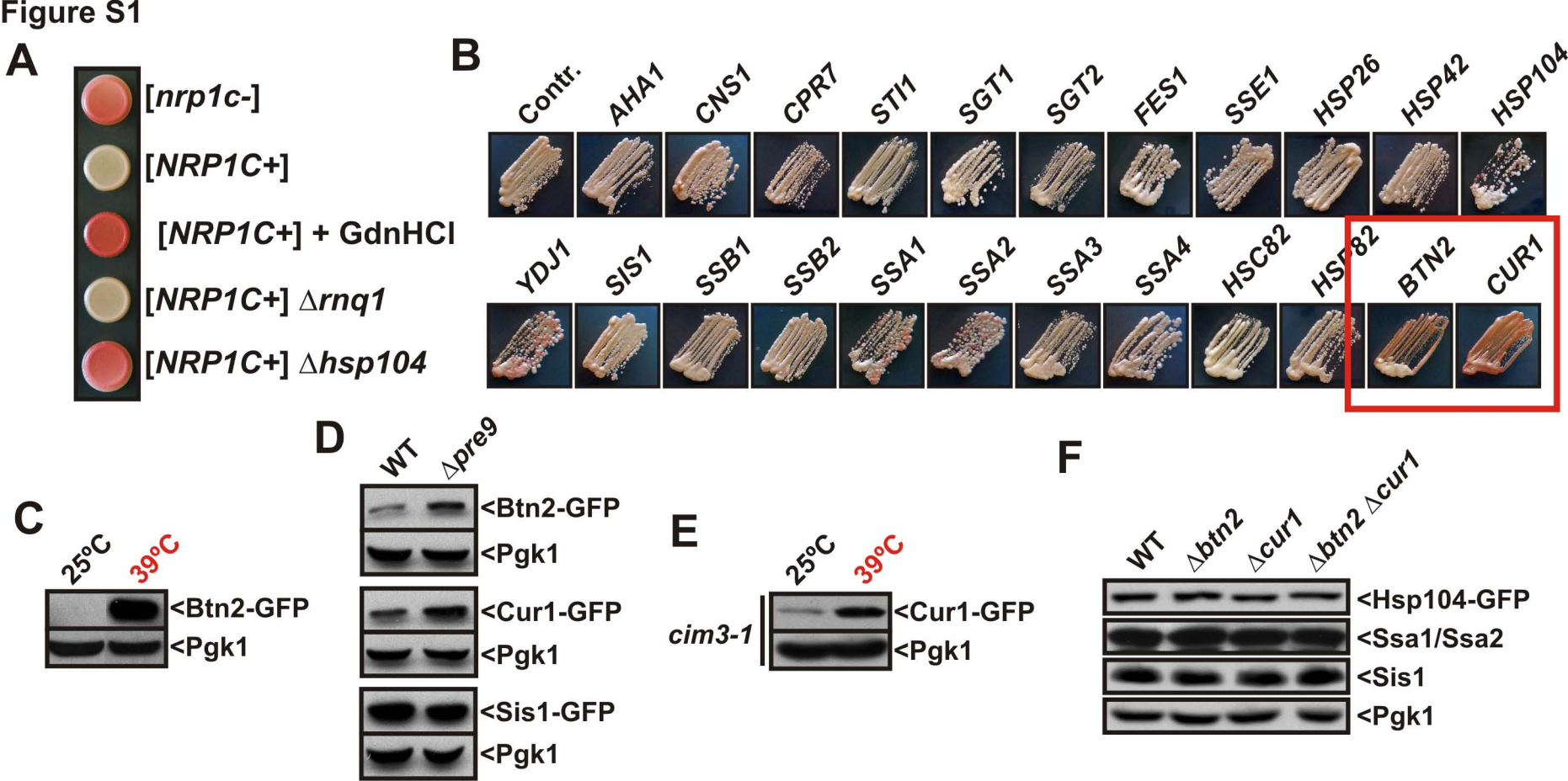

A
[nrp1c-]
[NRP1C+]
[NRP1C+] + GdnHCl
[NRP1C+] Δrnq1
[NRP1C+] Δhsp104
B
Contr.
AHA1
CNS1
CPR7
STI1
SGT1
SGT2
FES1
SSE1
HSP26
HSP42
HSP104
YDJ1
SIS1
SSB1
SSB2
SSA1
SSA2
SSA3
SSA4
HSC82
HSP82
BTN2
CUR1
C
25°C
39°C
<Btn2-GFP
<Pgk1
D
WT
Δpre9
<Btn2-GFP
<Pgk1
<Cur1-GFP
<Pgk1
<Sis1-GFP
<Pgk1
E
25°C
39°C
cim3-1
<Cur1-GFP
<Pgk1
F
WT
Δbtn2
Δcur1
Δbtn2 Δcur1
<Hsp104-GFP
<Ssa1/Ssa2
<Sis1
<Pgk1

Figure S2

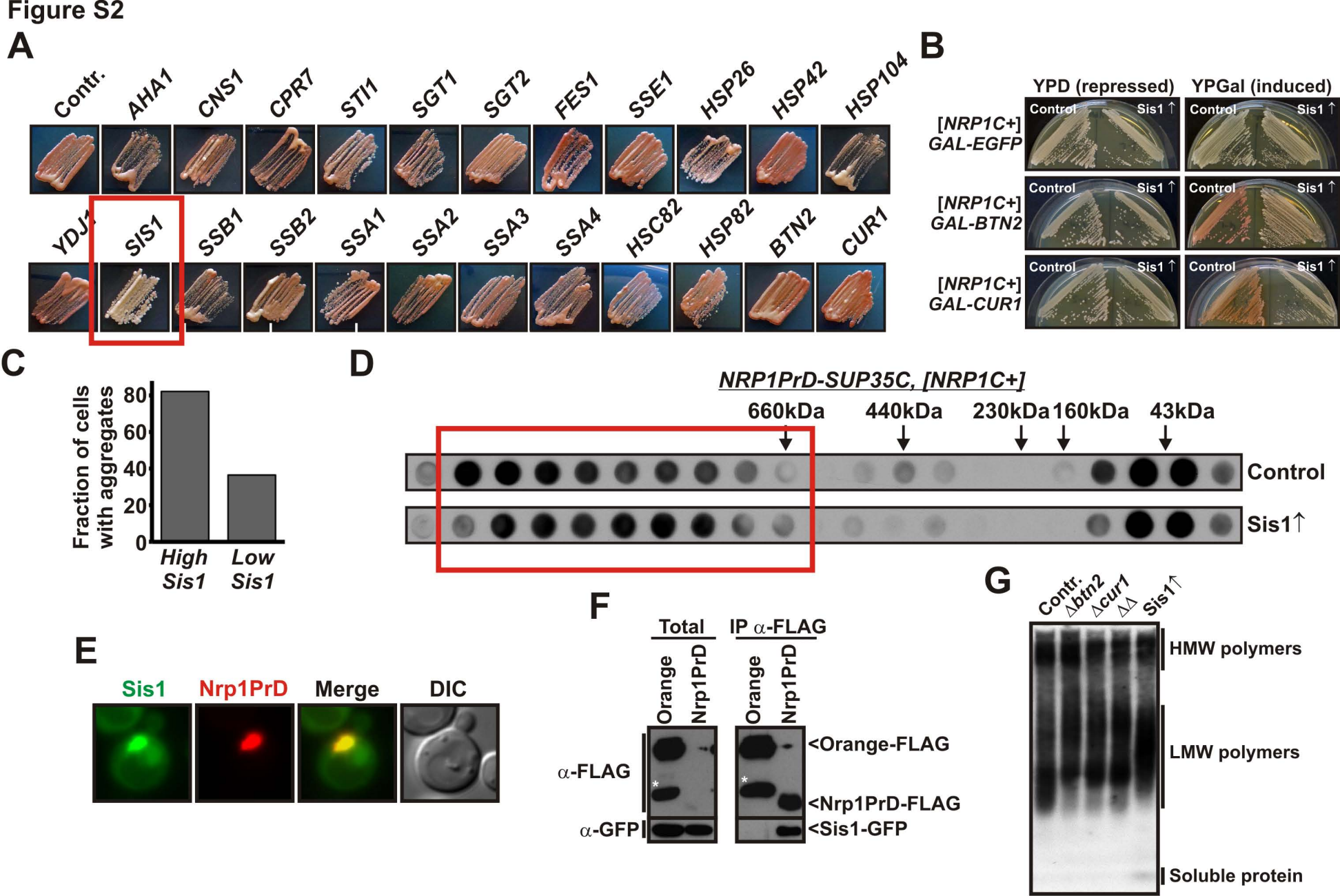

A
Contr.
AHA1
CNS1
CPR7
STI1
SGT1
SGT2
FES1
SSE1
HSP26
HSP42
HSP104
YDJ1
SIS1
SSB1
SSB2
SSA1
SSA2
SSA3
SSA4
HSC82
HSP82
BTN2
CUR1
B
YPD (repressed)
YPGal (induced)
[NRP1C+] GAL-EGFP
[NRP1C+] GAL-BTN2
[NRP1C+] GAL-CUR1
Control
Sis1↑
C
Fraction of cells with aggregates
80
60
40
20
0
High Sis1
Low Sis1
D
NRP1PrD-SUP35C, [NRP1C+]
660kDa
440kDa
230kDa
160kDa
43kDa
Control
Sis1↑
E
Sis1
Nrp1PrD
Merge
DIC
F
Total
IP α-FLAG
Orange
Nrp1PrD
α-FLAG
α-GFP
<Orange-FLAG
<Nrp1PrD-FLAG
<Sis1-GFP
G
Contr.
Δbtn2
Δcur1
ΔΔ
Sis1↑
HMW polymers
LMW polymers
Soluble protein

Figure S3

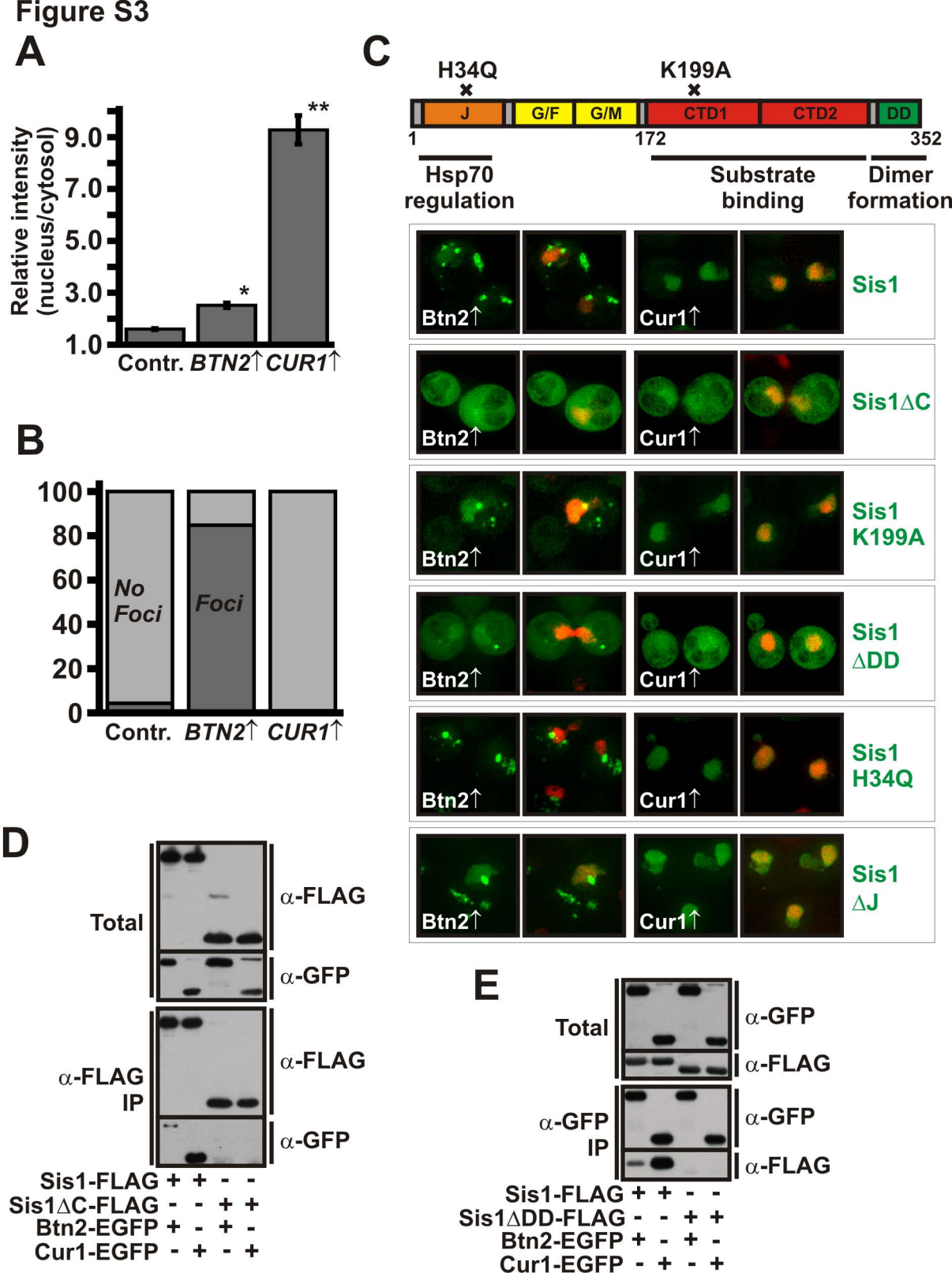

A
Relative intensity (nucleus/cytosol)
9.0
7.0
5.0
3.0
1.0
*
**
Contr. BTN2↑ CUR1↑
B
100
80
60
40
20
0
No Foci
Foci
Contr. BTN2↑ CUR1↑
C
H34Q
K199A
J
G/F
G/M
CTD1
CTD2
DD
1
172
352
Hsp70 regulation
Substrate binding
Dimer formation
Btn2↑
Cur1↑
Sis1
Sis1ΔC
Sis1 K199A
Sis1 ΔDD
Sis1 H34Q
Sis1 ΔJ
D
Total
α-FLAG
α-GFP
α-FLAG IP
α-FLAG
α-GFP
Sis1-FLAG + + - -
Sis1ΔC-FLAG - - + +
Btn2-EGFP + - + -
Cur1-EGFP - + - +
E
Total
α-GFP
α-FLAG
α-GFP IP
α-GFP
α-FLAG
Sis1-FLAG + + - -
Sis1ΔDD-FLAG - - + +
Btn2-EGFP + - + -
Cur1-EGFP - + - +

Figure S4

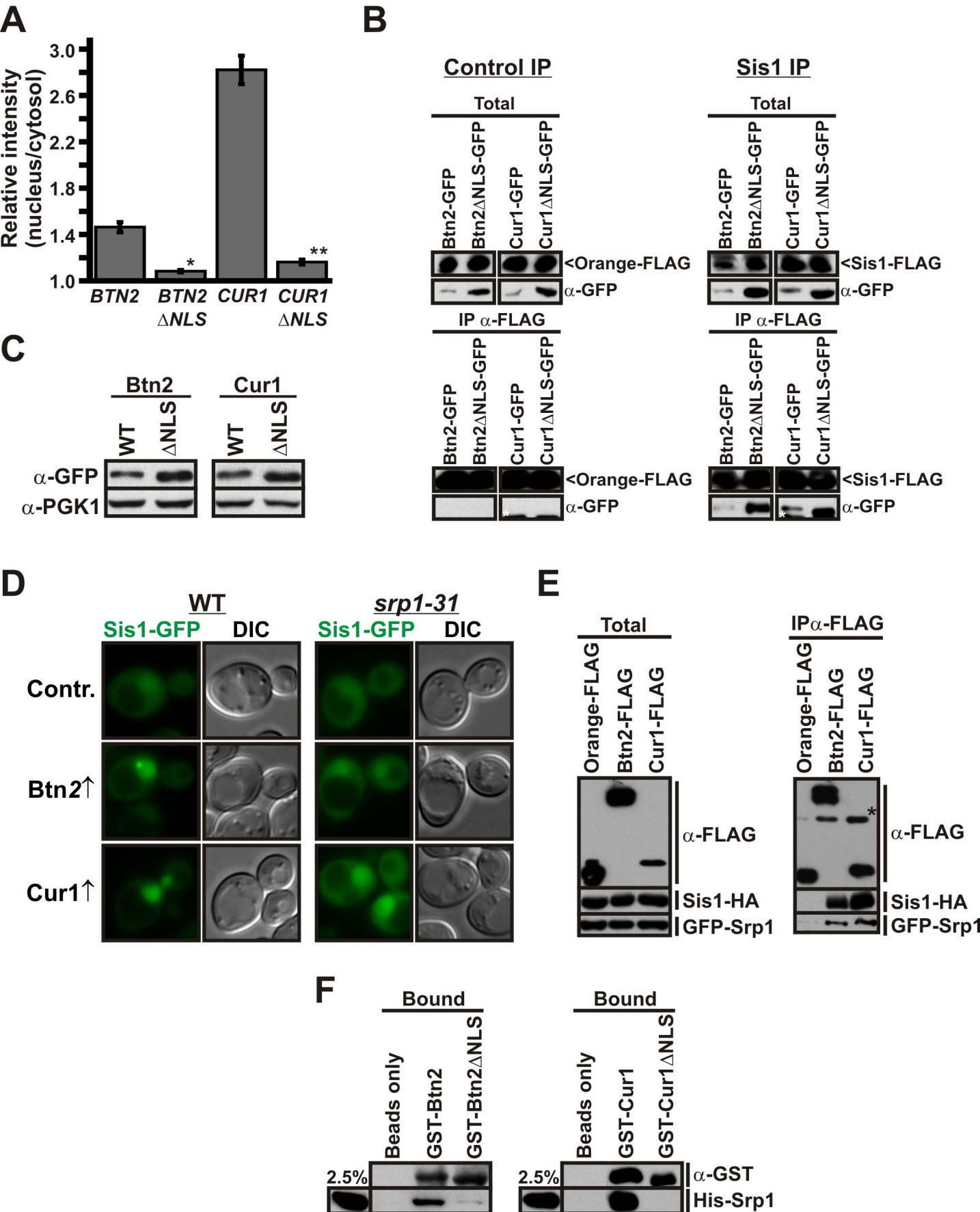

A
Relative intensity (nucleus/cytosol)
3.0
2.6
2.2
1.8
1.4
1.0
*
**
BTN2
BTN2 ΔNLS
CUR1
CUR1 ΔNLS
B
Control IP
Sis1 IP
Total
Btn2-GFP
Btn2ΔNLS-GFP
Cur1-GFP
Cur1ΔNLS-GFP
<Orange-FLAG
<Sis1-FLAG
α-GFP
IP α-FLAG
C
Btn2
Cur1
WT
ΔNLS
α-GFP
α-PGK1
D
WT
srp1-31
Sis1-GFP
DIC
Contr.
Btn2↑
Cur1↑
E
Total
IPα-FLAG
Orange-FLAG
Btn2-FLAG
Cur1-FLAG
α-FLAG
Sis1-HA
GFP-Srp1
F
Bound
Beads only
GST-Btn2
GST-Btn2ΔNLS
GST-Cur1
GST-Cur1ΔNLS
2.5%
α-GST
His-Srp1

Figure S5

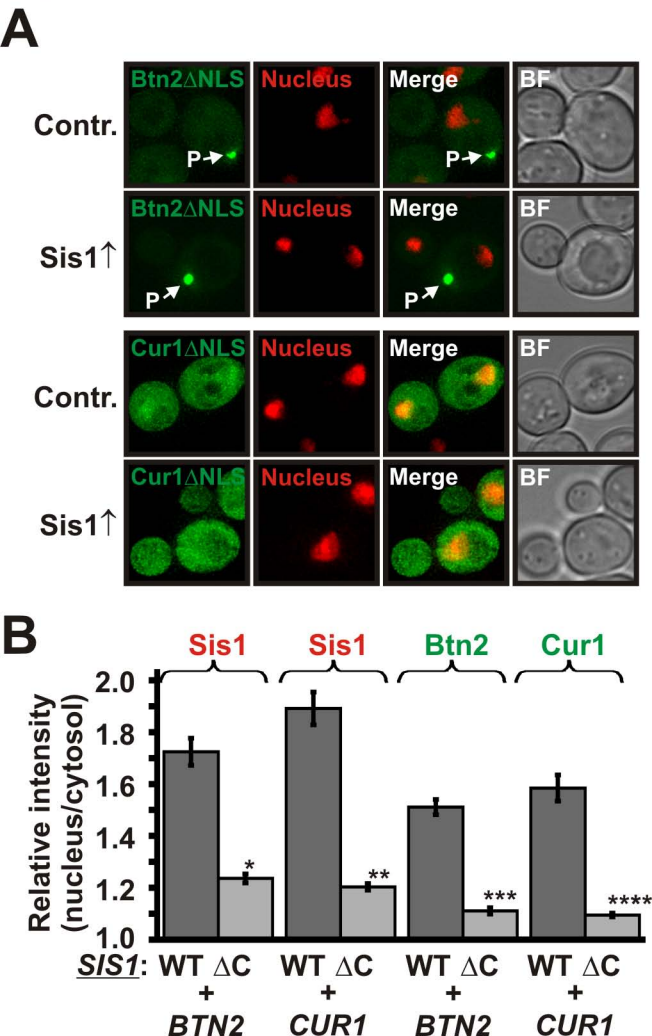

A
Btn2ΔNLS
Nucleus
Merge
BF
Contr.
Sis1↑
P
Cur1ΔNLS
B
Sis1
Sis1
Btn2
Cur1
Relative intensity (nucleus/cytosol)
2.0
1.8
1.6
1.4
1.2
1.0
*
**
***
****
SIS1: WT ΔC + BTN2
WT ΔC + CUR1
WT ΔC + BTN2
WT ΔC + CUR1

Figure S6

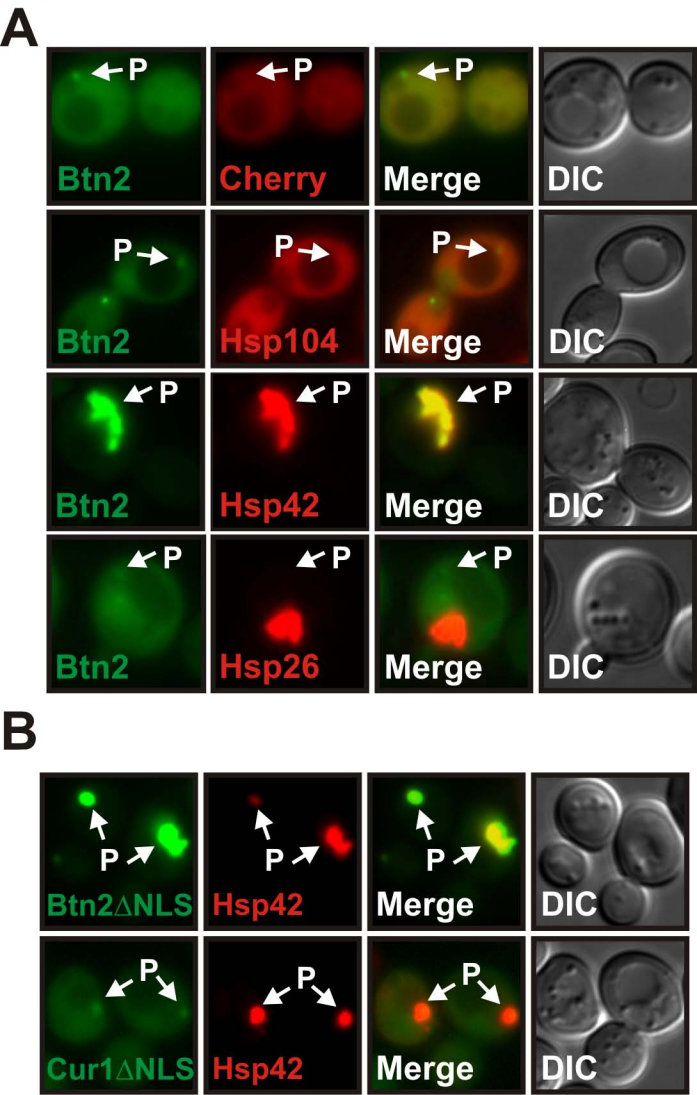

A
P
Btn2
Cherry
Merge
DIC
P
Btn2
Hsp104
Merge
DIC
P
Btn2
Hsp42
Merge
DIC
P
Btn2
Hsp26
Merge
DIC
B
P
Btn2ΔNLS
Hsp42
Merge
DIC
P
Cur1ΔNLS
Hsp42
Merge
DIC

Figure S7

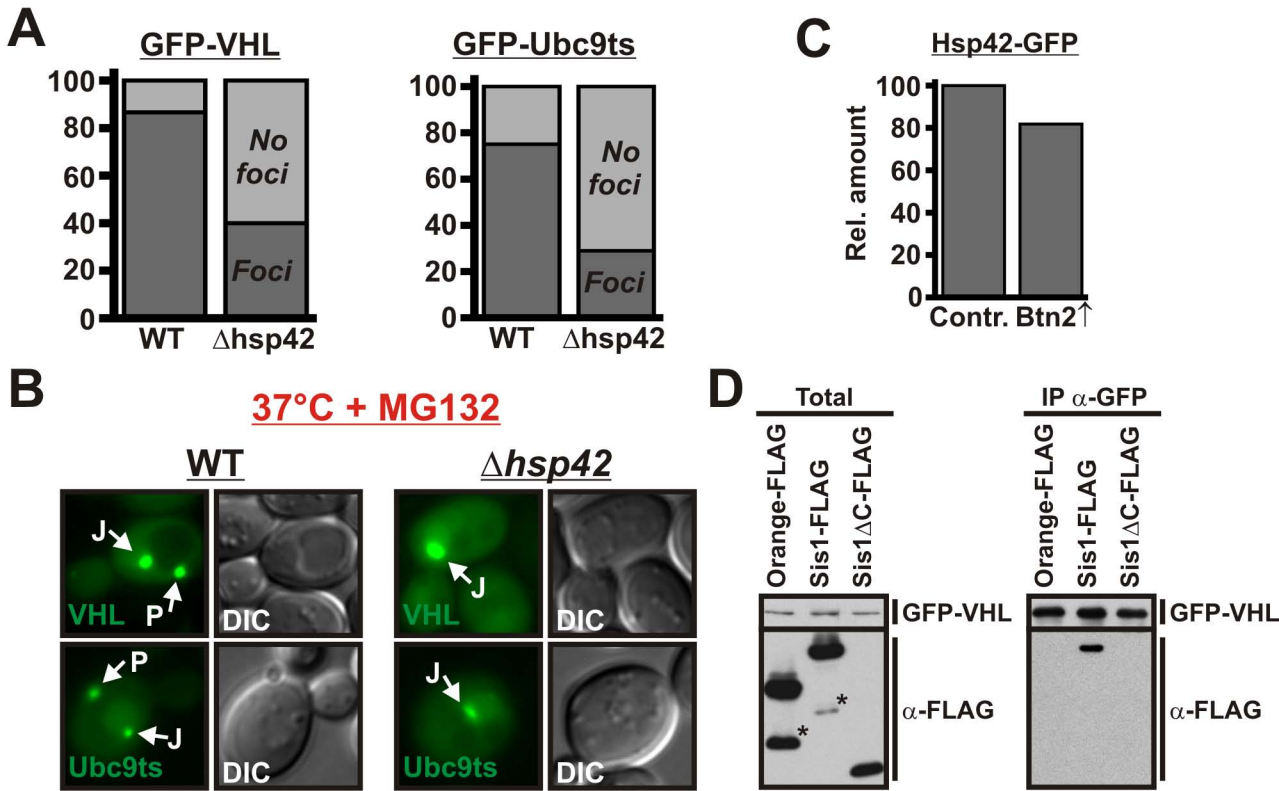

A
GFP-VHL
GFP-Ubc9ts
100
80
60
40
20
0
No foci
Foci
WT
Δhsp42
C
Hsp42-GFP
Rel. amount
Contr.
Btn2↑
B
37°C + MG132
WT
Δhsp42
J
P
VHL
Ubc9ts
DIC
D
Total
IP α-GFP
Orange-FLAG
Sis1-FLAG
Sis1ΔC-FLAG
GFP-VHL
α-FLAG

Figure S8

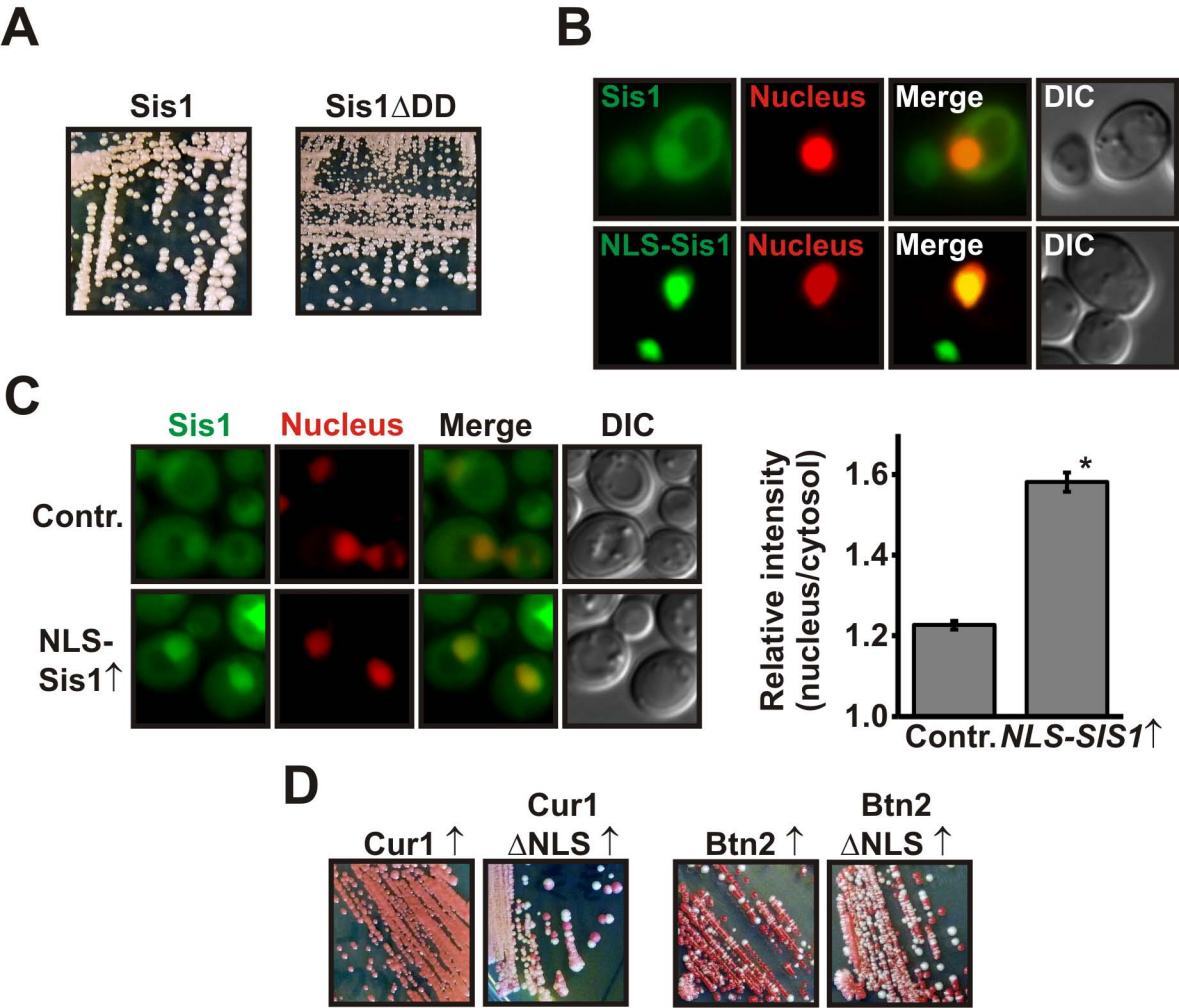
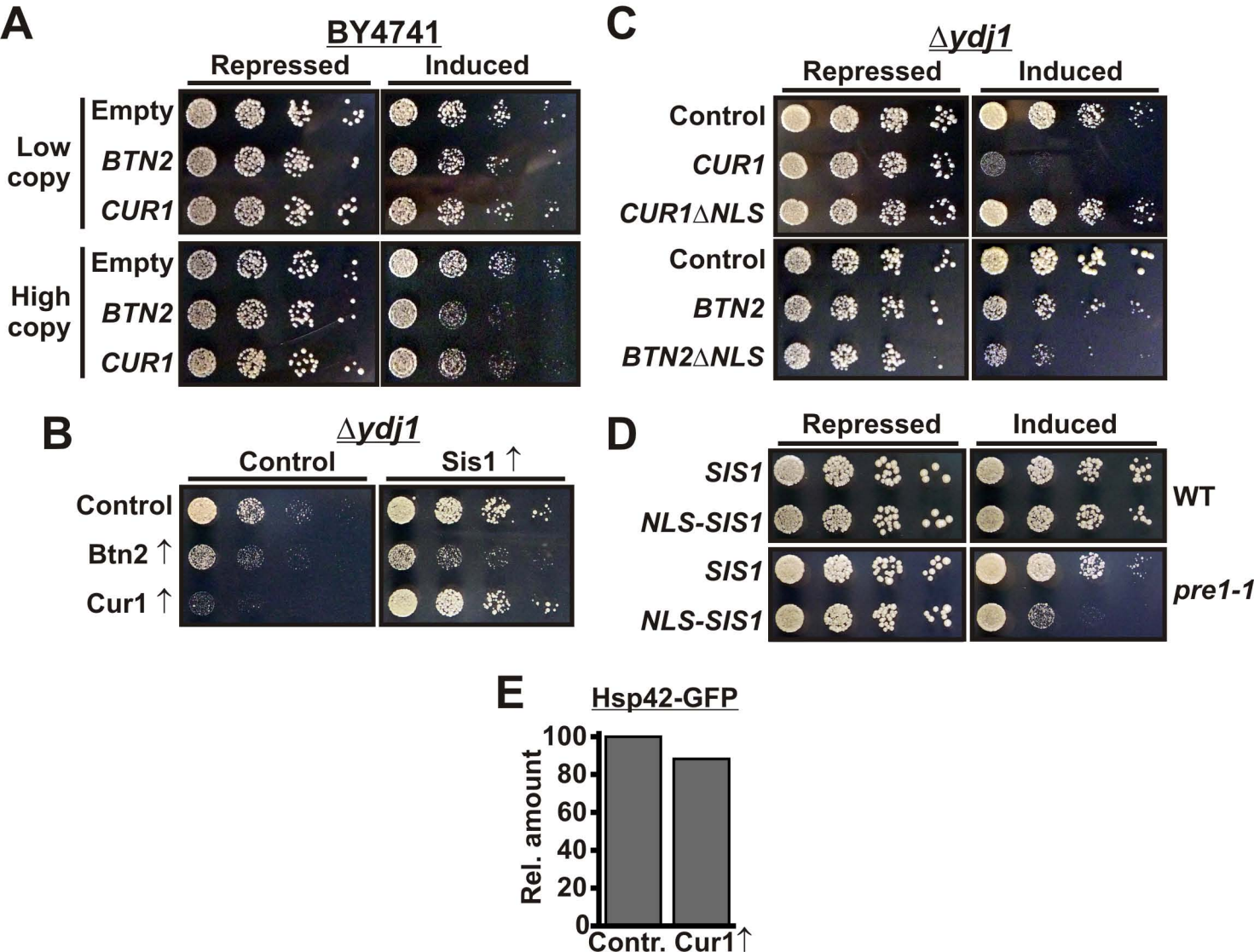

A
Sis1
Sis1ΔDD
B
Sis1
Nucleus
Merge
DIC
NLS-Sis1
Nucleus
Merge
DIC
C
Sis1
Nucleus
Merge
DIC
Contr.
NLS-Sis1↑
Relative intensity (nucleus/cytosol)
1.6
1.4
1.2
1.0
*
Contr.
NLS-SIS1↑
D
Cur1 ↑
Cur1 ΔNLS ↑
Btn2 ↑
Btn2 ΔNLS ↑

Figure S9


A
BY4741
Repressed
Induced
Low copy
Empty
BTN2
CUR1
High copy
Empty
BTN2
CUR1
B
Δydj1
Control
Sis1 ↑
Control
Btn2 ↑
Cur1 ↑
C
Δydj1
Repressed
Induced
Control
CUR1
CUR1ΔNLS
Control
BTN2
BTN2ΔNLS
D
Repressed
Induced
SIS1
NLS-SIS1
WT
SIS1
NLS-SIS1
pre1-1
E
Hsp42-GFP
Rel. amount
100
80
60
40
20
0
Contr.
Cur1↑